THE AMERICAN JOURNAL
OF PATHOLOGY

VOLUME VII SEPTEMBER, 1931 NUMBER 5

IMPLANTATION PERITONEAL CARCINOMATOSIS OF OVARIAN ORIGIN*

JOHN A. SAMPSON, M.D.

(From the Gynecological and Pathological Departments of the Albany Hospital and the Albany Medical College, Albany, N. Y.)

The purpose of this paper is to present the evidence that implantation peritoneal carcinomatosis of ovarian origin is nothing more than the result of the repair of injuries to the peritoneum caused by cancer cells which have escaped from ovarian tumors into the peritoneal cavity and lodged on the surface of its serous membrane, together with the continued growth of these cells in this situation.

The proof that carcinoma invades its host is absolute, because the direct extension of the former into the tissues of the latter can be definitely demonstrated.

Various ways have been described by which cancer cells, as emboli, may be conveyed from a primary neoplasm to nearby or distant structures and there develop into secondary tumors, *viz.*: the lymphatics, the blood stream, implantation by contact, implantation by cells escaping into cavities lined by serous membranes, implantation on epithelial-lined or covered surfaces and transplantation in the field of operation.

There is abundant support that carcinomata metastasize through the lymphatics. They are the channels by which the majority of these tumors are conceded to disseminate. The chain of evidence is most convincing, but it is broken and therefore the proof is not absolute. The same is true, in varying degree for the other mentioned means for the metastasis of cancer. The evidence in all is at best merely circumstantial. For this reason excellent pathologists

* Received for publication July 15, 1931.

may differ as to the method of origin of an assumed secondary tumor in a given case. Writers may readily misjudge the relative importance of the above mentioned ways for the dissemination of cancer and even question the validity of some of them.

In discussing the origin of peritoneal carcinomatosis, Ewing [1] states: "The most frequent source of these peritoneal growths is the ovarian adenocarcinoma which at any period may rupture its covering and be disseminated through the cavity. Its cells readily become implanted on the peritoneum, producing many miliary or large solid papillary or cystic tumors with a tendency to ascites."

On the other hand Karsner [2] states: "It is supposed that transfer to various parts of the peritoneum of a papilliferous adenocystoma of the ovary is by direct implantation of tumor fragments. This is probably true but cannot be regarded as finally proven because of the possibility that the dissemination is through the subperitoneal lymphatics."

While some pathologists undoubtedly agree with Karsner, Ewing (just quoted), Mallory,[3] MacCallum,[4] Kaufmann [5] and Boyd [6] concede, in their text-books, that certain ovarian tumors metastasize to the peritoneum by cells which escape from the primary growth into the peritoneal cavity and become implanted on the surface of the peritoneum. These pathologists are all well aware of the importance of the lymphatics as a means for the dissemination of cancer. On the other hand the pathologists, already mentioned, who believe in the implantation of cancer on the peritoneum have not described the steps in this process.

I have been able to find in the literature only one description of the implantation of cells from ovarian tumors on the peritoneum, and that by Hertzler.[7] He states: "In papillary serous ovarian cysts often the cyst wall has ruptured allowing the tumor mass to become exposed, particles break off and scatter over the peritoneum and there become implanted. These heal in just as a foreign body does. Their presence excites a reaction on the part of the peritoneum which in turn exudes its plastic material and a fibrin network covers the tumor tissue. Growth then continues." Hertzler's description is an excellent "text" and a true one, but it is not complete. In justice to the subject it should be elaborated and a more detailed description given.

The problem of the implantation of cancer cells on the peritoneum is a combination of the behavior of the peritoneum to small foreign bodies escaping into that cavity and lodging on its serous lining, and of tissue culture — especially the growth of epithelium in skin grafting. Some of the cancer cells are likely to be alive and just as capable of growth as are those escaping into the lymphatics and blood stream. We are not concerned with the site, avenues and means by which foreign material is absorbed from the peritoneal cavity (for a review of the literature of this phase of the subject see an excellent article by Cunningham[8]), but with the fate of foreign material retained in that cavity. Hertzler [9] has reviewed the literature on the results of injecting suspensions of pigment into the peritoneal cavity of the lower animals and includes the results of his own studies on this subject. He states that "this is followed by an increased exudation into the peritoneal cavity. With this increase of exudation there is a sweeping of the particles toward the nooks and dependent portions of the peritoneal cavity and about the great omentum. This may take place in any direction quite as pronouncedly toward the pelvis as toward the diaphragm." The foreign bodies float about until they become anchored on the peritoneum by fibrin, which is transformed into fibrous tissue. Thus the foreign body is eventually covered or encapsulated by the peritoneum.

Neuhof [10] in his monograph, The Transplantation of Tissues, reviews the literature on this subject and in describing the histology of autografts of skin (Thiersch graft) states: "There first occurs an exudate of fibrin fixing the transplant in place. This becomes infiltrated with leucocytes and fibroblasts and disappears gradually to be replaced by a richly vascular connective tissue. About fourteen days after transplantation the granulation tissue has changed into an organized membrane."

It can be seen that the reaction of the peritoneum to a dead foreign body is quite similar to that of the tissue receiving the skin graft, except for one very important feature. The tissues of the peritoneum grow over and cover the foreign body while the skin graft remains uncovered. In both instances we are dealing with the repair of injured tissues. The general laws or principles governing the repair of tissue injuries are the same, irrespective of the agent causing the injury, the nature or degree of the latter, its complications or the tissue injured. All these tend to heal after the manner of wounds.

As emphasized by Mallory,[11] "the underlying principles are the same." We may divide the repair of tissue injury or the healing of wounds into three general stages.

1. *The fixation stage.* Mall [12] showed that the first step in the healing of anastomoses of dogs' intestines is the union of the apposed serous surfaces by fibrin. It is "the basting suture" uniting approximated tissue in wounds and likewise the scaffolding or temporary framework spanning gaps caused by loss of tissue or failure of approximation. Fibrin anchors foreign bodies in wounded tissue and on the surface of the peritoneum. Fibrin also temporarily holds the skin graft in place.

2. *The organizing stage.* Fibroblasts alone, or both fibroblasts and vascular endothelium grow out into the fibrin and make the union of the apposed surfaces of wounds firmer. When there is loss of tissue or gaps caused by failure of approximation, they invade the fibrin in these gaps, giving rise to granulation tissue.

3. *The organized or healed stage.* The fibrin invaded by fibroblasts or replaced by granulation tissue is converted into scar tissue, the final step in the healing of a wound, the encapsulation of foreign bodies, the formation of adhesions and the permanent "taking" of a skin graft.

Material and Methods of Study

The material which is the basis of the present study was obtained from twenty-five cases of peritoneal carcinomatosis associated with ovarian cancer, operated upon in the gynecological department of the Albany Hospital during the last nine years. It is of very little statistical value because of the small number of cases, the lack of uniformity with which the material was chosen and studied, and the necessarily incomplete study of all lesions possibly present in any operative case. A very important feature of such a problem is the examination of the cellular contents of the fluid present in these cases. This was done in only six instances.

In all but three cases both tubes and ovaries and the uterus were removed at operation, and in some of these also bits of parietal peritoneum, an epiploical appendage, the appendix or a portion of the omentum. But this relatively small amount of material does not enable one to determine the distribution of all the metastases or to

study all types of lesions possibly present. In only two instances was a postmortem examination obtained. In neither of these were the pelvic organs removed at operation. In one an exploratory incision was made, but on account of the great extent of the cancer no attempt was made to remove the primary tumors. In the other a cecostomy was done to relieve intestinal obstruction. In both of these cases metastases were present on the under surface of the diaphragm. At operation one is not able to determine whether or not the diaphragm is involved.

An epiploical appendage was removed at operation in sixteen cases by carefully grasping the base of the appendage by two fine clamps, cutting between them, ligating the stump and immediately placing the severed appendage in formalin without handling it. The appendix, removed in thirteen cases, was also carefully handled in order not to injure its peritoneal surface. Interesting portions of the specimen removed at operation were carefully excised at the close of the operation and placed in formalin. In all instances routine examinations were made of the rest of the specimen removed, but the results of such examinations are unfortunately too often of little value in the study of special problems. The majority of the material was hardened in formalin, embedded in celloidin, and the sections stained with hematoxylin and weak eosin. They were faintly stained with eosin in order to lessen the difficulty in obtaining satisfactory photomicrographs. In many instances serial sections were made in order to determine the exact condition present. This is of the greatest importance, otherwise what appears to be an early implant in a single section may prove to be the advancing edge of a large one, if serial sections are made.

I believe that the study of epiploical appendages and appendices, removed and fixed as just described, is of great value, not only in eliminating trauma and obtaining absolutely fresh material, but also in better determining the possible source of the peritoneal metastases on these structures. It is a short step and an easy one for cancer cells escaping from the ovary into the peritoneal cavity to land, with the assistance of the fluid present in these cases, on the surface of nearby epiploical appendages, the appendix, or even distant structures such as the under surface of the diaphragm. On the other hand, it is a long jaunt, and often over a route difficult to explain, for cancer cells to reach these situations through the lymphatics or blood stream

from an ovarian cancer. In lesions involving the peritoneum of the tubes and uterus, a direct extension from the primary tumor, or retrograde metastases through the lymphatics and venous circulation common to these organs, can sometimes be more readily defended.

The cellular contents of the ascitic fluid from six of these cases was studied. A large amount of fluid was centrifugalized, the supernatant fluid poured off and replaced by formalin, thus fixing the sediment. The sediment, when fixed, was removed from the tube, tied in a small piece of gauze and embedded in celloidin. It was then removed from the gauze, mounted, cut and stained with hematoxylin and eosin. In this way we were able accurately to compare the cellular contents of this fluid with those of the primary and secondary tumors.

The oldest patient was 82, and the youngest 38 years of age. Fifteen were over 50 and only two under 40 years of age.

I am unable properly to classify all of the ovarian tumors. Twenty can be grouped as either carcinomatous ovarian cysts or cystic adenocarcinomata. Of these twenty cases, fifteen were bilateral. Of the solid ovarian tumors, only five in number, four were bilateral.

In all instances many peritoneal metastases were present. These were usually most abundant in the posterior cul-de-sac. The frequent infiltration of the peritoneum with cancer in this situation is an important feature in the diagnosis of peritoneal carcinomatosis. Free fluid was present in all cases, and in the majority of them in sufficient amount to cause abdominal distention. Omental metastases were described, in the operative notes, as being present in eighteen cases. In only two instances was the omentum described as appearing normal, while in the remaining five cases no mention was made of its condition, an unfortunate omission. In only a few cases was a portion of the omentum excised for microscopic study. As has been shown in experimental work on the lower animals, the omentum is the great net for picking up foreign particles introduced into the peritoneal cavities of these animals.

To report the results of the microscopic examination of the material obtained from all of the twenty-five patients would at best represent only a small fraction of all conditions actually present in these cases. I trust that a study of the conditions observed in the few cases which have been chosen as the most instructive and repre-

sentative, together with the illustrations and their legends, may stimulate others to make further and better studies of peritoneal carcinomatosis. It has so influenced me.

Report of Cases

It is well recognized that foreign material, which escapes into the peritoneal cavity and is not absorbed, becomes encapsulated. The process of encapsulation has been already described. It consists of the fixation of the foreign body by fibrin, the invasion of the fibrin by fibroblasts alone, or by both fibroblasts and vascular endothelium, and finally the replacement of this condition by scar tissue. The results of this process are well shown in the findings of the following case (Case 1).

Case 1. The patient, a physician, aged 54 years, was suddenly seized with severe abdominal pains, November 21, 1916, while making morning rounds in the Albany Hospital. He remained in the hospital eleven days and was discharged with a diagnosis of acute cholecystitis. This was his first and only acute illness. On June 15, 1918, nearly nineteen months later, he was operated upon after a diagnosis of chronic cholecystitis was made. At operation the gall-bladder was found to be normal, but the distal portion of the stomach, as well as the first part of the duodenum, was bound down by old adhesions. On these adhesions, as well as on the anterior surface of the stomach, were small, opaque nodules resembling metastatic cancer. The appendix was removed, a posterior gastro-enterostomy made, and one of the small nodules on the anterior wall of the stomach was excised. A microscopic examination of the nodule showed it to be encapsulated food (Fig. 1) from an evident perforation of a duodenal ulcer, November 21, 1916. The patient died suddenly December 7, 1922. At postmortem an occlusion of the anterior descending branch of the left coronary artery was found. Small nodules on the peritoneum, which were observed at operation, were still present. One of these, removed at the time of the postmortem from the anterior surface of the right lobe of the liver, is shown in Fig. 2. Food of various kinds, such as bread, peas, corn, oatmeal, fruit and lettuce were fixed in Zenker's solution, cut and stained with the same technique as the nodules removed at operation and at postmortem. The histological structure of the encapsulated foreign bodies just described was iden-

tical with that of the cooked oatmeal, and also with that shown in the photomicrographs of a similar case reported by Shennan.[13] I subsequently learned that the physician's breakfast, for many years, had consisted of fruit, oatmeal and cream, an egg, toast and a cup of coffee.

CASE 2. The patient, aged 71 years, had an extensive peritoneal carcinomatosis, most marked in the posterior cul-de-sac and in the omentum, associated with cystic adenocarcinoma of both ovaries. A large amount of ascitic fluid was present. The abdominal distention with the physical signs of free fluid, the induration in the posterior cul-de-sac, and a palpable, movable tumor (omental cake) in the midabdomen enabled us to make a correct preoperative diagnosis. At operation, September 18, 1930, a large amount of fluid was obtained, and both tubes and ovaries and an epiploical appendage removed. The ovarian tumors were of about the same size (7 to 8 cm. in their greatest diameters). They were adherent to the posterior layer of the broad ligaments and presented gross evidence that the cancer had extended through their capsules. The histological structure of a carcinomatous metastasis of the peritoneum of the epiploical appendage is shown in Fig. 3. Note its close resemblance to the encapsulated food which had escaped through the perforation of a duodenal ulcer and lodged on the anterior wall of the stomach and the surface of the liver (Case 1, Figs. 1 and 2). The ascitic fluid obtained at operation was centrifugalized and the sediment fixed, embedded in celloidin and cut and stained. The microscopic examination of sections of this sediment showed large masses of mesothelial cells and also clumps of hyperchromatic cells (Fig. 4). The latter were similar to those of the primary tumor and the metastatic growth of the peritoneum of the epiploical appendage. The evidence, strong though only circumstantial, would indicate that the metastatic nodule of the epiploical appendage arose from the encapsulation (implantation) of similar cells becoming attached to the peritoneal surface of the appendage, just as food escaping through a perforated duodenal ulcer became implanted on the peritoneum (Case 1).

CASE 3. The patient, aged 42 years, had extensive peritoneal carcinomatosis, most marked in the omentum, associated with solid alveolar carcinoma of both ovaries. Ascitic fluid was present, but the amount was not as great as in the preceding case. The correct

preoperative diagnosis was considered. At operation, October 15, 1930, fluid was obtained and both tubes and ovaries, the entire uterus and an epiploical appendage removed. The ovaries were of about the same size (6 to 7 cm. in their greatest diameter) and were adherent to the posterior surfaces of the broad ligament and the uterus. The ovarian tumor had invaded the lymphatics of that organ and also had pierced its capsule (see Fig. 5). Clumps of hyperchromatic cells, indistinguishable from those in the ovarian tumor and with an arrangement similar to the latter, were present in the sediment obtained from the centrifugalized ascitic fluid (Fig. 6). Multinuclear giant cells also were present in both situations. Peritoneal lesions in various stages of development were present on the surface of the tubes and uterus. Similar lesions were also present on the peritoneum of the epiploical appendage. One of these is shown in Fig. 7. Clumps of cells, with a histological structure and arrangement identical with those in the ovarian tumor and in the ascitic fluid, are enmeshed in granulation tissue. This represents the organizing stage in the reaction of the peritoneum to injury from foreign bodies lodging on it surface. Cancer was not found in the deeper tissues of the epiploical appendages. The invasion of the lymphatics of the ovary by cancer indicates that it had or might have metastasized through these channels. The exposure of parts of the tumor to the peritoneal cavity, the presence of similar cells in the ascitic fluid, the well recognized reactions of the peritoneum to injuries caused by foreign bodies lodging on its surface, and the inclusion of cancer cells in these reactions indicate that some of the cancer had been disseminated in this way. The patient was greatly relieved by the operation, but this relief was only temporary.

CASE 4. The patient, aged 41 years, had extensive peritoneal carcinomatosis, most marked in the posterior cul-de-sac and omentum, associated with bilateral ovarian carcinoma. A very large amount of ascitic fluid was present. A correct preoperative diagnosis was made. At operation September 26, 1923, both tubes and ovaries, the uterus, an epiploical appendage and a piece of the parietal peritoneum were removed. Unfortunately ascitic fluid was not saved for a study of its cellular contents. Both ovaries were of about the same size and presented similar conditions. They were adherent by their lateral surfaces to the posterior layers of the broad ligaments. The cancer evidently had developed on their lateral and under surfaces

and had only superficially invaded the deeper tissues of these organs (Fig. 8). The ovarian cancer presented the appearance of having been of implantation origin or having arisen from the surface "epithelium" of the ovaries. I believe the latter. Cancer was not found in the lymphatics of the ovaries. The histological structure of the surface of the ovarian tumors, exposed to the peritoneal cavity, demonstrated the ease with which cancer cells could be discharged from these tumors into that cavity (Figs. 9 and 10). Every stage in the reaction of the peritoneum to foreign bodies lodging on its surface, and the encapsulation of the cancer cells (the healing of wounds caused by these) was found (see Figs. 11, 12, 13, 14, 15 and 16). These could all be interpreted as similar stages in the implantation of cancer cells which had escaped from the primary tumor and lodged on the surface of the peritoneum. In the peritoneal metastasis on the epiploical appendage (Fig. 18), the manner of the spread of the cancer over the surface of the appendage is clearly shown, as well as the invasion of its deeper tissues and the possibility of further dissemination of cancer cells to the peritoneal cavity from the surface of exposed portions of the growth. The direct extension of cancer from the peritoneal growths into the underlying tissues was present in many situations (Fig. 16, 18 and 51). In a few situations cancer was found in the peritoneal and subperitoneal lymphatics (Fig. 51). As every metastatic tumor has the same potentialities of invasion and dissemination as a primary tumor in its situation, the presence of cancer in the lymphatics near such a growth could more readily have been derived from cancer near it than from a distant primary tumor. The patient was greatly relieved by the operation, but this relief was only temporary.

CASE 5. Patient, aged 53 years, had a very extensive peritoneal carcinomatosis, most marked in the posterior cul-de-sac and in the omentum, associated with bilateral ovarian carcinomata. A large amount of ascitic fluid was present. A correct preoperative diagnosis was easily made. At operation July 22, 1922, the uterus, both tubes and ovaries, the appendix and epiploical appendage were removed. Ascitic fluid was not saved for a study of its cellular contents. Both ovaries were of about the same size and presented similar conditions. They were both adherent by their lateral surfaces to the posterior layers of the broad ligaments. The histological structure of the surface of these ovarian tumors, exposed to the peritoneal

cavity, demonstrated the ease with which cancer cells might be discharged from the tumor into that cavity (Fig. 20). As in the preceding case, every stage in the reaction of the peritoneum to injury from foreign bodies (cancer cells) lodging on its surface, and the repair of these injuries, was present (see Figs. 21, 22, 23, 24, 25, 26 and 27). All these can be interpreted as similar stages in the implantation of cancer on the peritoneum. Many, but not all, possible phases of the subsequent life history of these peritoneal metastases were found. The direct invasion of underlying tissue (Figs. 19 and 26), necrosis with ulceration (Fig. 19), encapsulation and death (Fig 19) were present. On the other hand, cancer was not definitely found in the lymph vessels in any of the sections, as in the preceding case. Of special interest is the peritoneal metastasis shown in Fig. 27. It has the appearance of cancer arising from the mesothelium. I believe that it is more likely an implant without encapsulation, similar to a successful skin graft. The patient was greatly relieved by the operation, subsequently had radium and deep X-ray treatment, but died.

CASE 6. Patient, aged 63 years, had a papillary adenocystic-carcinoma of both ovaries, and an adenocarcinoma of the mucosa of the body of the uterus, histologically indistinguishable from that in the ovaries; associated with a general peritoneal reaction (granulation tissue stage). There were enmeshed in this granulation tissue occasional clumps of epithelium-like cells with the same histological appearance and arrangement as those of the ovarian tumors. No fully organized peritoneal metastases were found. A large amount of ascitic fluid was present. A correct preoperative diagnosis was not made. At operation June 29, 1923, the entire uterus, both tubes and ovaries were removed, and also the appendix, an epiploical appendage and a piece of the omentum. Ascitic fluid was not saved for a study of its cellular contents. Both ovarian tumors were of the same size and presented similar conditions (Fig. 28). The ovarian tumors were not adherent. The gross appearance of these tumors (Fig. 28), as well as their histological structure (Fig. 29), demonstrates that both cancer cells and any secretion from the growth might readily escape into the peritoneal cavity. Microscopic examination of sections of the tubes (Fig. 32), uterus, epiploical appendage (Figs. 30 and 33) and the appendix (Fig. 31) demonstrated granulation tissue on the peritoneal surface of the same. In a few places there were

enmeshed in this granulation tissue clumps of cells with an adenomatous arrangement histologically identical with those of the ovarian tumor. We must account for the reaction of the peritoneum to some irritant resulting in the development of granulation tissue, with epithelium-like cells enmeshed in it.

The conditions shown in Figs. 28 and 29 indicate a portal of entry into the peritoneal cavity for both secretions from the ovarian tumors and also malignant cells. Had the patient not been operated upon at this time we would have missed a very important stage in the development of peritoneal carcinomatosis. The patient was seen six months later and presented the physical signs of an extensive peritoneal carcinomatosis, namely — free fluid, marked induration in the cul-de-sac, and a mass in the region of the omentum. Cancer was not found in the lymphatics in any of the sections. I believe that the carcinomata of both ovaries and the body of the uterus were probably independent tumors — an instance of multicentric origin.

Case 7. The patient, aged 82 years, had an extensive peritoneal carcinomatosis with the greatest involvement in the pelvis and omentum, associated with a cystic adenocarcinoma of both ovaries, the right being the larger and apparently primary tumor. Ascitic fluid was present. The patient was admitted to the Albany Hospital October 6, 1930, with the symptoms of intestinal obstruction. X-rays, after a barium enema, revealed that an obstruction was situated in the sigmoid. An exploratory laparotomy was done with the intention of making a colostomy above the site of obstruction. This was found to be impossible on account of the marked infiltration of the mesocolon with cancer, and a cecostomy was done. The patient died October 21, 1930. At postmortem the following conditions were found: A multilocular adenocarcinocystoma of the right ovary (Fig. 40) which was adherent to the surrounding structures and with gross evidence, in many places, of either an extension of the cancer through the walls of loculi of the cyst or else a rupture of some of these. A marked infiltration of the pelvic peritoneum and organs with cancer was present. The left ovary was only slightly enlarged, but was almost entirely replaced by cancer of the same structure as that in the right ovary, except that the loculi were smaller. The carcinoma in the left ovary had apparently invaded that organ, through its hilum from cancer involving the tissues of the left broad ligament

(Fig. 41). Peritoneal metastases of various ages were scattered throughout the abdominal cavity, especially involving the intestinal tract. There was a large metastasis in the omentum. Many small metastases, apparently of different ages, were present on the under surface of the diaphragm (Figs. 42, 43, 44 and 45). The retroperitoneal and mediastinal lymph nodes, which were carefully examined, showed no metastases. With such an extensive involvement of the pelvic organs and the peritoneum and intestinal tract, one would expect to find metastases to the retroperitoneal lymph nodes. All conditions observed in this case can readily be explained by the escape of cancer cells from the primary tumor, their subsequent implantation on the peritoneum and invasion of underlying structures.

Discussion

I am well aware of the evidence indicating that ovarian cancer, at times, metastasizes through the lymphatics and blood vessels. I have seen cancer cells in these vessels in carcinomatous ovaries and have encountered metastases in the uterus, tubes, and in other situations in patients with apparent primary ovarian carcinoma which could best be explained as having arisen through these channels. I have found metastases in the lymph nodes of only four patients with ovarian cancer. In two of these the peritoneum was free from cancer. In the other two a typical extensive peritoneal carcinomatosis was present. The lymph node containing cancer, in each of the latter cases, was from the groin. It is possible that the cancer cells causing the metastases to the inguinal nodes in these cases might have arisen from the invasion of lymphatics by cancer implanted on the peritoneum about the round ligament or internal ring on that side, rather than from the primary ovarian tumors (bilateral in both cases). I have had the opportunity of studying the abdominal and pelvic lymph nodes of only two patients dying with advanced peritoneal carcinomatosis associated with ovarian cancer. One of these cases is reported in this paper (Case 7). We were unable to find cancer in any of the lymph nodes from these two patients.

I do not deny the possibility of peritoneal metastases arising from an ovarian tumor by way of the lymph or blood streams, but have never seen a peritoneal metastasis in situations other than the fim-

briated portion of the tube, which I thought was due to this phenomenon. I believe that they must be of infrequent occurrence as compared with peritoneal metastases arising from the implantation of cells escaping into the peritoneal cavity. It is often difficult to determine the exact method of origin of advanced secondary tumors. The vast majority of the early lesions of peritoneal carcinomatosis which I have studied have presented conditions similar to those caused by injury to the peritoneum from foreign bodies lodging on its surface, and the various stages in the repair of these injuries. When I have observed cancer extending from a metastatic peritoneal growth into the tissues beneath it, or cancer in nearby lymphatics, I have usually considered these a manifestation of invasion and dissemination from the metastatic tumor, rather than an indication of permeation or metastasis from the primary ovarian cancer. This applies particularly to lesions of the peritoneum, of epiploical appendages, appendices and diaphragm, and not to all lesions involving the peritoneum of the tubes and uterus.

From a study of the material which has been presented in this paper, the life history of peritoneal carcinomatous implants of ovarian origin may be divided into the following stages:

1. The escape of cancer cells into the peritoneal cavity from the ovarian tumor.
2. The transportation of these cells to their site of implantation.
3. The reaction of the peritoneal tissue injured by the cancer cells lodging on its surface.
   (*a*) Fixation of the cancer cells by fibrin.
   (*b*) Organization of the fibrin.
   (*c*) The organized implant, the various types and their subsequent behavior.

Cells apparently escape into the peritoneal cavity from a variety of ovarian cancers, not only from cancer arising on the surface of the ovary but also from that starting within that organ and extending through to its surface, and from the rupture of the walls of cystic adenocarcinomata. A study of the histological structure of the portion of these cancers which is exposed to the peritoneal cavity will convince one that this phenomenon must take place (Figs. 5, 8, 9, 10, 20, 28, 29 and 40). This same study indicates that sometimes this may occur early and that the discharge of cancer cells into the peritoneal cavity from some of these tumors may be of frequent

occurrence, or even more or less constant. These cells would usually escape into the pelvic cavity and most frequently lodge in the posterior cul-de-sac. There is often a varying amount of free fluid in the peritoneal cavity, even in patients with essentially normal pelvic organs. I have been collecting this fluid and studying its cellular content for several years and especially the last year. When the amount is small, most of it settles in the pelvis irrespective of the normal posture of the patient. It is often possible at operation on the pelvic organs of women to scoop up with a ladle which I use for this purpose 10 cc. or more of this fluid from the posterior cul-de-sac. Cancer cells escaping from the ovarian tumor would float about in this fluid like the foreign bodies experimentally introduced into the peritoneal cavities of the lower animals, and in time would settle on some portion of the peritoneum. Their presence, in intimate contact with the peritoneum, in some way irritates the latter, with a resulting exudate which increases the amount of fluid already present.

As previously stated, a histological study of the ovarian tumors should convince us that cancer cells escape into the peritoneal cavity from this source. A similar study of the cellular contents of the ascitic fluid obtained from these patients demonstrates in many instances clumps of cells identical in form, arrangement and staining qualities with those of the ovarian tumor, and totally unlike the desquamated mesothelial cells present in the same fluid (Figs. 4, 6 and 49), and also unlike cells found in the ascitic fluid of patients without an ovarian cancer. I believe that the cells which have escaped from ovarian cancers into the peritoneal cavity often continue to multiply in that fluid like bacteria in a fluid culture medium. The clumps of cells frequently are larger than one imagines would break off or be discharged from the primary growth, stain as intensely as those of the primary tumor, and occasionally mitotic figures are present. The fluid arising from the peritoneal irritation caused by cancer cells escaping into the peritoneal cavity is the most important factor in the transportation of these cells. The latter go wherever the fluid goes. The amount of fluid, gravity, and the posture of the patient determine the situation of the fluid and the presence of its cellular contents. When the fluid is small in amount a large part of it remains in the pelvic cavity. When the amount is large it has no difficulty in reaching all parts of the abdominal cavity including the diaphragm, with the patient in the recumbent pos-

ture. Therefore, the amount of fluid determines, in large measure, the distribution of the metastases. The bottom of the pelvis is exposed to the cellular contents of this fluid earlier and longer than any other part of the peritoneal cavity. It is here that apparently the oldest lesions and the most marked infiltration of the peritoneum with cancer are usually found.

It has been shown in experimental work on the lower animals that a large amount of the foreign material introduced into the peritoneal cavities of these animals becomes enmeshed in the omentum. The omentum of patients with peritoneal carcinomatosis is often markedly infiltrated with cancer.

As is well known, a skin graft will not "take" unless the surface to be grafted is suited for the reception of the former. The cancer cells float about in the fluid and sooner or later some of them lodge on the surface of the peritoneum. Their presence irritates (injures) the peritoneum. This is followed by an exudate and in places a denudation of the peritoneum (casting off of the mesothelium). Fibrin forms on the surface of the injured peritoneum. A study of the very early peritoneal lesions suggests that the cause of the injury inflicted upon the peritoneum by the cancer cells might be more than that of an inert foreign body. Possibly there may be a toxin or enzyme present which escapes from the cancer cells and increases the irritation. It is also possible that the ascitic fluid may sometimes contain this toxin in solution. This might be derived from the cancer cells floating about in the fluid, and possibly also from the primary growth when the contents of a cystic tumor escape into the peritoneal cavity, or from cancer which is exposed to that cavity. The reason for believing that the fluid may contain a soluble irritant is that the peritoneal injury in some cases appears to be much greater than one which can arise from the small number of cancer cells in actual contact with the peritoneum. Next to the cancer cells themselves, fibrin is the most important factor in the life history of these implants (Figs. 11, 12 and 14). It is fibrin which holds the cancer cells in place by splinting them against the denuded peritoneum. The cancer cells may become enmeshed in fibrin which has arched over portions of the peritoneum or dangles from the latter like a fringe. Fibrin is both the temporary framework and the scaffolding of the organized implant, and in large measure determines the form of the latter.

The fibrin becomes organized by fibroblasts growing out through breaks in the surface of the injured peritoneum and invading the fibrin (Figs. 11, 12 and 14). Sometimes organization apparently occurs with the assistance of fibroblasts alone. In other instances vascular endothelium also invades the fibrin, which with the fibroblasts forms granulation tissue (Figs. 15, 30, 31, 33 and 34).

The fully organized implant arises from the transformation of fibrin invaded by fibroblasts, or of granulation tissue into connective tissue. It is the result of the repair of the peritoneum injured by cancer cells lodging on its surface (the scar of the peritoneal wound). Its form is determined by many factors — the condition of the surface of the peritoneum at the time of the fixation of the cancer cells; the amount of fibrin and its arrangement (the architecture of the scaffolding and temporary framework); the situation of the cancer cells in this design, whether beneath the fibrin, enmeshed in it or on its surface; the type of the organization of the fibrin (whether by fibroblasts alone or both by fibroblasts and vascular endothelium), and its age and the activity of the cancer cells.

Often the mature implant consists of cancer cells embedded in the thickened peritoneum (Figs. 3, 13, 24, 26, 44, 45 and 48). In other instances it resembles a polyp wholly or partially encapsulated; the polyp may be sessile and when so the base may be solid (Figs. 16, 23, 38, 39, 51, 54 and 56) or fenestrated (Fig. 23); occasionally the polyp is attached to the peritoneum by a very slender pedicle (Figs. 17 and 37). Implantations in adhesions frequently occur (Figs. 7, 33, 34, 35 and 36). All of these forms, just described, are the results of the repair of the peritoneum injured by cancer cells lodging on its surface.

There is one type of peritoneal cancer found in patients with peritoneal carcinomatosis for which a metastatic origin might be questioned, and that is the non-embedded or non-encapsulated growth. This is attached to or spreads over the surface of the peritoneum like a primary tumor which had arisen from the mesothelium in this situation (an instance of the multicentric origin of cancer). In my experience it is of infrequent occurrence as compared with the other types already described. Excellent examples of this type of growth are shown in Figs. 24, 27, 42, 46 and 47. It is not peculiar to any one variety of ovarian cancer and is found with other types of implants in the same patient. Its development apparently is not determined

by the organ on which it is situated (see the illustrations just mentioned). It manifests itself as a papillary tumor or spreads over the peritoneum with a single layer of cells as though re-covering it. I have designated this uncovered metastasis as an implantation of the skin graft type. Originally the cancer cells might have become attached in a manner similar to a skin graft, or more likely the cancer cells lodging on the peritoneum were first covered by fibrin which did not become organized by fibroblasts and therefore disappeared. It is also possible that even if the fibrin covering it had become organized the growth of the cancer may subsequently have destroyed the latter.

I believe that only occasionally do the cancer cells of an implant die. I have observed a few instances of this. Although encapsulation often checks the growth of these cells so that they may remain dormant for a long time, its efforts are too often futile. There is a constant struggle between the cancer and the tissues of its host, the cancer to invade and its surrounding tissue to check that invasion. Polypoid implants with a sessile base may gradually flatten. This is caused by the invasion of the structures beneath it, the lateral expansion of the tumor, and the attempt of the surrounding tissue to encapsulate it (compare Fig. 16 with Fig. 51, and Fig. 54 with Fig. 56).

As a general rule cancer implanted on the peritoneum has the same potentialities of invasion and dissemination as a primary tumor. It invades the peritoneum and underlying structures (see Figs. 16, 18, 19, 20, 26, 38, 39, 41, 45, 48, 51, 52, 53, 54, 55, 56 and 58). It gains access to lymph vessels (Figs. 51, 55, 56, 58 and 59) and probably metastasizes through them. The conditions shown in Figs. 18, 24, 27, 46 and 47 indicate that cancer cells escape into the peritoneal cavity from such implants as well as from the primary tumor, and may give rise to secondary implantations of cancer on the peritoneum.

Patients with ovarian carcinoma usually do not die from the primary growth, but from its implants by the direct extension and dissemination of cancer cells from these.

Bilateral ovarian cancer was found in nineteen of the twenty-five cases of peritoneal carcinomatosis. The question naturally arises, were these ovarian cancers independent tumors or was one secondary to the other? When the tumors are of the same or nearly of the

same size, one is tempted to believe that they might be independent tumors (multicentric origin). When there is a marked difference in their size, one may have been derived from the other, or the development of the cancer in one of the ovaries may have been deferred. A study of the peritoneal surfaces of malignant ovarian tumors demonstrates implantation-like lesions in various stages of development which have a histological structure identical with the peritoneal implants in other situations in the same patient.

For this reason I believe that sometimes one ovarian cancer may be derived from the other by the implantation of cancer cells on its surface. I have seen only one instance in which the cancer of the second ovary was very small. It presented the histological structure of an implant of the skin graft type (not encapsulated), and therefore was indistinguishable from a cancer arising from the surface epithelium of the ovary.

The infiltration of the omentum with cancer is usually so great that my studies of its development in this situation have been unsatisfactory. I would expect it to present the same stages as implantations elsewhere in the peritoneal cavity.

It is obvious that there are many problems of both scientific interest and clinical importance associated with implantation peritoneal carcinomatosis of ovarian origin.

Why are patients apparently benefited by the removal of the pelvic organs? It is true that the relief is usually only temporary. All but three of the twenty-five patients mentioned in this paper are dead. The three living ones were operated upon within a year and a half. By removal of the primary tumors we often remove only one source of the dissemination of cancer cells into the peritoneal cavity. It is true that the majority of implants are encapsulated, but some are not, and even in those which are, the cancer, by direct extension, often pierces the capsule.

Do cancer cells escaping into the peritoneal cavity actually multiply like bacteria in a fluid culture medium? I believe they do. How long will they live in this medium without implantation? Is the irritation of the peritoneum by these cells solely that of a foreign body or is there some toxin in these cells which adds to the injury? If there is a toxin, may it not also be in solution in the ascitic fluid?

Is the malignancy of the cancer cells in the implant greater or less than that of those in the ovarian tumor? Can secondary implanta-

tions of cancer actually arise from the implants? Apparently they can.

Does the peritoneum of patients with peritoneal carcinomatosis actually develop a relative immunity to the implantation of cancer on its surface? I believe that this occurs in some instances.

What can be done to increase this immunity and likewise the encapsulation of the cancer cells, and retardation of the growth of these cells? Will deep X-ray therapy and radium accomplish this?

Is the removal of the omentum of any value? These and many other questions have occurred to me while studying this subject.

Conclusions

Implantation peritoneal carcinomatosis arises from the repair of injuries to the peritoneum caused by cancer cells which have escaped into the peritoneal cavity and lodged on the surface of its serous membrane, together with the continued growth of these cells in this situation.

The various stages in this repair, as well as the laws governing the same, are similar to those encountered in the repair of tissues injured by foreign bodies, and in the taking of skin grafts — namely, the healing of wounds.

The histological structure of these implants varies with the reaction of the peritoneal tissues before and after the fixation of the cancer cells and the activity of the latter.

As a result cancer becomes embedded in the peritoneal scar, encapsulated on its surface, enmeshed in adhesions, or like the epithelial growth of a successful skin graft spreads over the peritoneum without encapsulation.

The malignant cells of these metastatic tumors possess the same potentialities of invasion and dissemination as those of a primary cancer.

Note: The laboratory work on this paper was greatly facilitated by the technical skill and care of Miss Isabel Peck.

The illustrations of the gross specimens and the coloring of the photomicrographs were made by Mrs. M. R. Marden, and the photomicrographs by Mr. James A. Glenn. These I thank for their interest and coöperation.

REFERENCES

1. Ewing, James. Neoplastic Diseases. W. B. Saunders Co., 1928, 81.
2. Karsner, H. T. Human Pathology. J. B. Lippincott Co., 1929, 335.
3. Mallory, F. B. The Principles of Pathologic Histology. W. B. Saunders Co., 1929, 395.
4. MacCallum, W. G. A Text-Book of Pathology. W. B. Saunders Co., 1928, 1080.
5. Kaufmann, Edward. Pathology for Students and Practitioners. P. Blakiston's Son & Co., 1929, **2**, 878.
6. Boyd, William. Surgical Pathology. W. B. Saunders Co., 1929, 585.
7. Hertzler, A. E. The Peritoneum. C. V. Mosby Co., 1919, 812.
8. Cunningham, R. S. The physiology of the serous membranes. *Physiol. Rev.*, 1926, **6**, 242–280.
9. Hertzler, A. E. The Peritoneum. C. V. Mosby Co., 1919, 14, 15, 253 and 255.
10. Neuhof, Harold. The Transplantation of Tissues. D. Appleton & Co., 1923, 37 and 38.
11. Mallory, F. B. The Principles of Pathologic Histology. W. B. Saunders Co., 1929, 74.
12. Mall, F. Healing of intestinal sutures. *Johns Hopkins Hosp. Rep.*, 1896, **1**, 76–92.
13. Shennan, T. Foreign-body tubercles on serous coat of stomach, caused by escape of particles of oat-seed. *J. Path. & Bact.*, 1923, **26**, 82.

DESCRIPTION OF PLATES

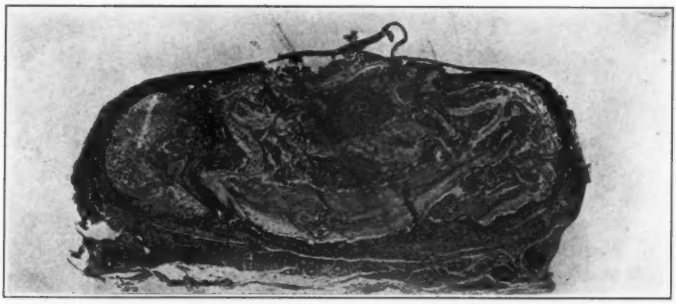
PLATE 82

FIG. 1. Photomicrograph of encapsulated food found, at operation, on the anterior wall of the stomach of a patient who had a perforation of a duodenal ulcer, one and one-half years before (Case 1). The foreign body is entirely encapsulated and strands of connective tissue, like trabeculae or the stroma of a cancer, have developed between portions of it. This represents the results of the repair of injury to the peritoneum caused by a particle of food lodging on its surface. × 25.

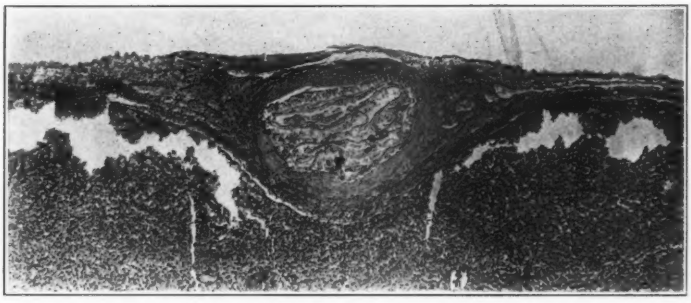
FIG. 2. Photomicrograph of food embedded in the capsule of the anterior surface of the liver, obtained at postmortem (Case 1), four and one-half years after the operation mentioned in Fig. 1. The clinical history of the case indicates that this food escaped into the peritoneal cavity six years before death. The histological structure of the food in this section is identical with that shown in Fig. 1 and also with that of cooked oatmeal fixed in Zenker's solution, cut and stained with hematoxylin and eosin, as was the encapsulated food shown in this and the preceding illustration. × 25.

Fig. 3. Photomicrograph of a metastatic cancer of the peritoneum of an epiploical appendage from a patient with peritoneal carcinomatosis associated with bilateral ovarian cancer (Case 2). Histologically it is in many ways identical with the final stages in the healing of the wounds of the peritoneum caused by food escaping into the peritoneal cavity, shown in Figs. 1 and 2. It differs from the latter in that the cells of this encapsulated foreign body have grown and invaded its capsule. It represents either cancer arising in the peritoneum independent of the ovarian cancer (multicentric origin) or, if derived from the latter, it must have been from cancer cells transported to this situation through the blood or lymph vessels, or by the encapsulation (implantation) of cells escaping into the peritoneal cavity from the ovarian tumors. Cancer was not found in the lymphatics of the appendage. × 25.

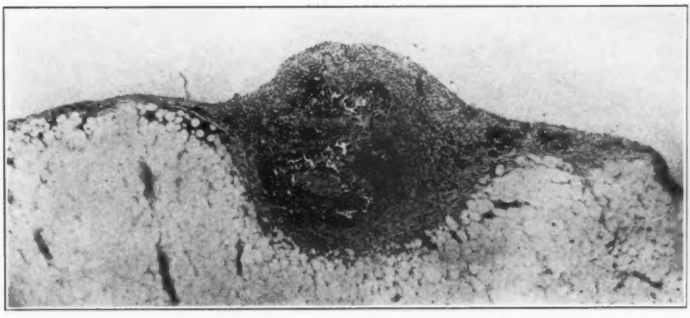
FIG. 4. Photomicrograph of a section of the sediment from centrifugalized ascitic fluid obtained from Case 2. I believe that the paler cells were derived from the mesothelium but the clumps of hyperchromatic cells, similar to those in the ovarian tumors, as well as those in the metastatic nodule shown in Fig. 3, might have escaped into the peritoneal cavity from the primary tumors. Could the condition shown in Fig. 3 possibly have arisen from the repair of injury to the peritoneum of the epiploical appendage by similar cells lodging on its surface, just as food escaping into the peritoneal cavity became encapsulated on or embedded in the peritoneum (Figs. 1 and 2)? × 60.



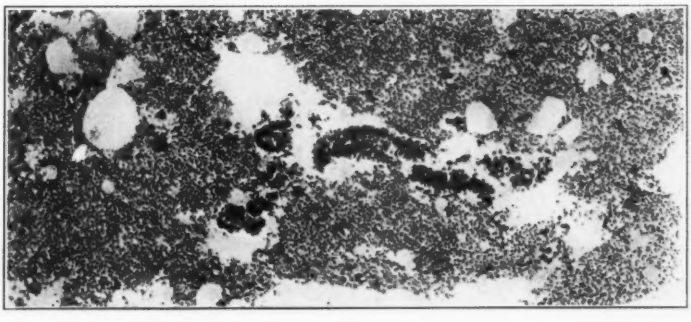
1



2



3



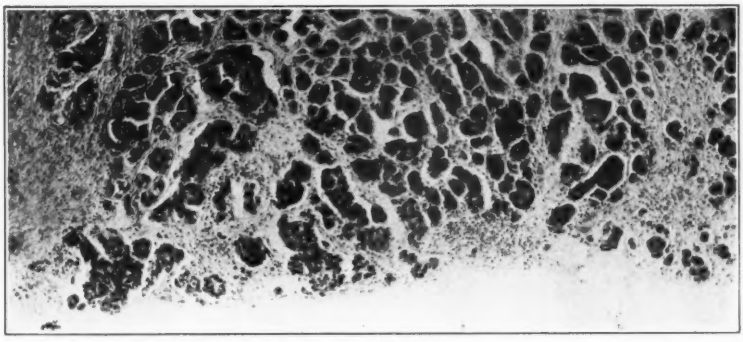
4

PLATE 83

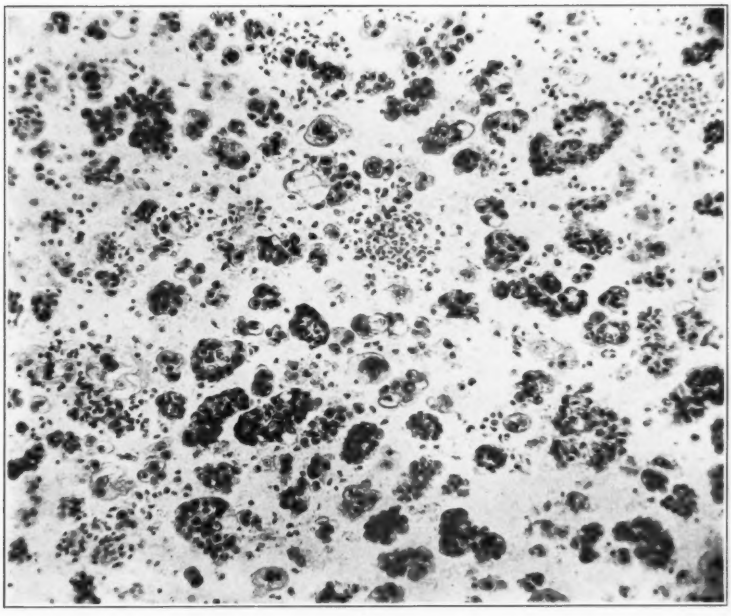
FIG. 5. Photomicrograph of a section through a portion of the surface of an ovary of a patient with peritoneal carcinomatosis associated with bilateral ovarian cancer (Case 3). It is conceivable that cancer cells might easily escape into the peritoneal cavity from such an area. If so we would expect to find them in the ascitic fluid (Fig. 6). Should any of these cells wound the peritoneum we would expect to find evidence of such an injury (see Fig. 7). × 60.

FIG. 6. Photomicrograph of a section of the sediment from the centrifugalized ascitic fluid obtained from Case 3. Clumps of hyperchromatic cells are present, with an appearance and arrangement identical with those shown in Fig. 5. Multinuclear giant cells are also present as well as clumps of smaller and paler cells similar to the mesothelial cells shown in Fig. 3. Were the larger, hyperchromatic cells derived from a metaplasia of the peritoneal mesothelium or from cancer cells discharged from the ovary shown above? Is there evidence that similar cells might have injured the peritoneum as foreign bodies injure this structure? × 130.

FIG. 7. Photomicrograph of a section through the surface of an epiploical appendage (Case 3). Granulation tissue, a well recognized reaction to tissue injury, has arisen on the peritoneal surface of the appendage and enmeshed in this tissue are clumps of cells identical in appearance and arrangement with those of the ovarian cancer (Fig. 5), as well as with the cells of the sediment from the centrifugalized fluid (Fig. 6). The condition is similar to the organizing stage in the repair of injury to the peritoneum from foreign bodies escaping into the peritoneal cavity. Might it not also represent a similar stage in the repair of a like injury to the peritoneum caused by cancer cells which had escaped from the ovarian tumor, as indicated in Figs. 5 and 6? × 60.



5



6

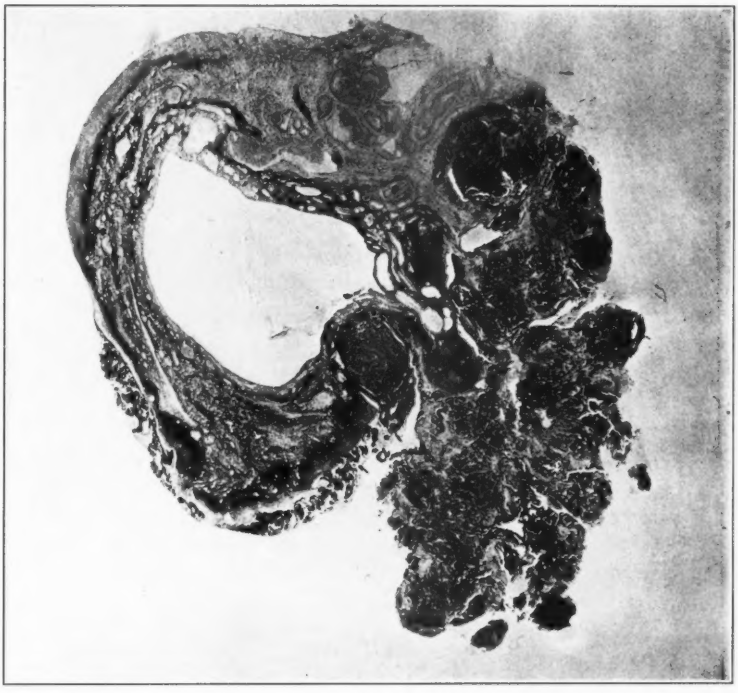
7

Sampson Implantation Peritoneal Carcinomatosis

PLATE 84

FIG. 8. Photomicrograph of a cross-section of the right ovary from a patient with peritoneal carcinomatosis, associated with bilateral ovarian cancer (Case 4). The cancer has invaded the ovary from its peritoneal surface and is most marked on the lateral surface which was fused with the posterior layer of the broad ligament. The under surface of the ovary was free and here cancer can be seen arising from, or replacing the surface epithelium. (See Fig. 9.) × 5.

FIG. 9. Photomicrograph of a portion (a) of the under surface of the ovary shown in the preceding illustration. The origin of the cancer from the surface epithelium or the replacement of the latter by the growth is well shown. One can understand how cancer cells might easily escape into the peritoneal cavity from this portion of the growth. Unfortunately ascitic fluid, in this case, was not saved for a study of its cellular contents. × 60.



8

9

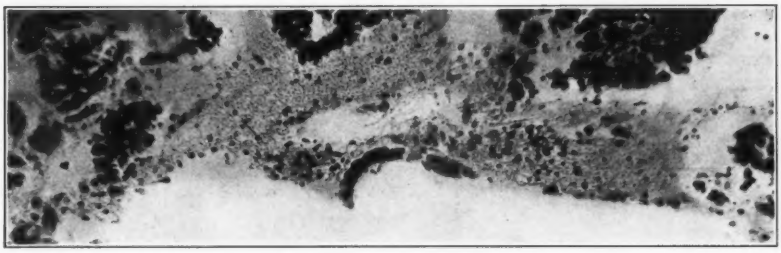
PLATE 85

FIG. 10. Photomicrograph of the surface of the cancer shown in the preceding illustration, again emphasizing the ease with which malignant cells might escape into the peritoneal cavity. × 130.

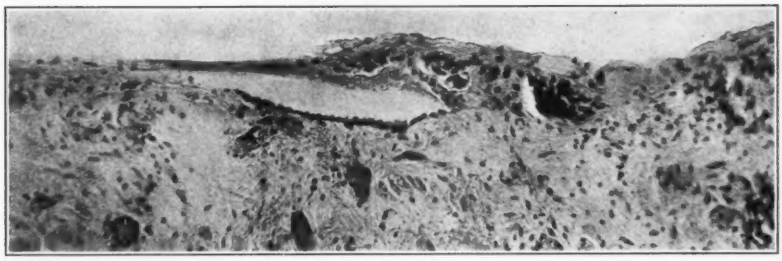
FIG. 11. Photomicrograph of a section through the posterior surface of the mesosalpinx (Case 4). The peritoneum, evidently, has been recently injured and shows a characteristic reaction. The mesothelium has been cast off from the peritoneum except in the center. Here fibrin has arched over the mesothelium, later to become organized and form a mesothelial inclusion. The surface of the denuded peritoneum is covered with fibrin. Clumps of epithelium-like cells are enmeshed in this fibrin. Were these cells derived from the cast-off mesothelium or from the cancer shown in Fig. 10? Histologically they are indistinguishable from the latter. The process, shown here, is identical with that of the fixation stage in the encapsulation of small foreign bodies experimentally introduced into the peritoneal cavities of the lower animals. Could it not also represent a similar stage in the implantation of cancer cells which we realize must have escaped into the peritoneal cavity? × 130.

FIG. 12. Photomicrograph also through the posterior layer of the mesosalpinx but in a different location from that shown in Fig. 11. The entire surface of this portion of the peritoneum is denuded of mesothelium and covered by fibrin which has been infiltrated with cells growing in from the underlying tissue. The fibrin with its cellular contents has arched over a clump of epithelium-like cells deposited in a crevice of the mesosalpinx. This could readily represent the onset of the organizing stage in the implantation of cancer, similar to a like stage in the encapsulation of a foreign body, namely — the cellular infiltration of the fibrin covering a foreign body. × 130.

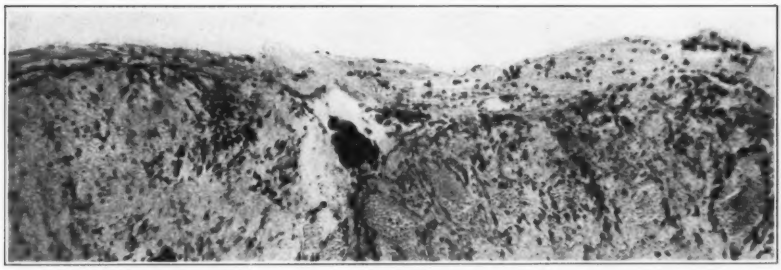
FIG. 13. Photomicrograph of a section through the parietal peritoneum (Case 4). The surface of this portion of the peritoneum is denuded of mesothelium. The peritoneum is thickened and infiltrated with cells presenting the well known picture of irritation of long duration (compare with Figs. 11 and 12). Embedded in the thickened peritoneum are two clumps of epithelium-like cells identical in structure with those shown in the two preceding illustrations. It could well represent the organized stage in the implantation of cancer, identical with a similar stage in the healing of a peritoneal injury with the encapsulation of the foreign body causing the injury. × 130.



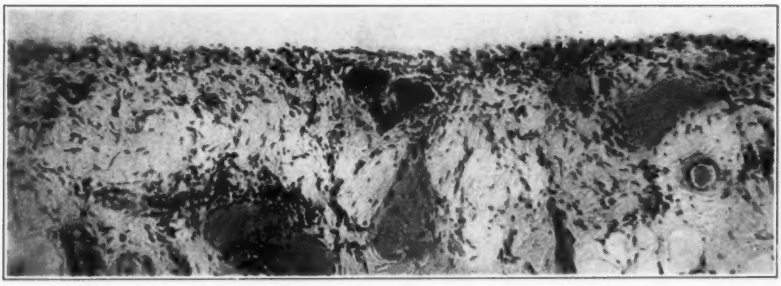
10



11



12

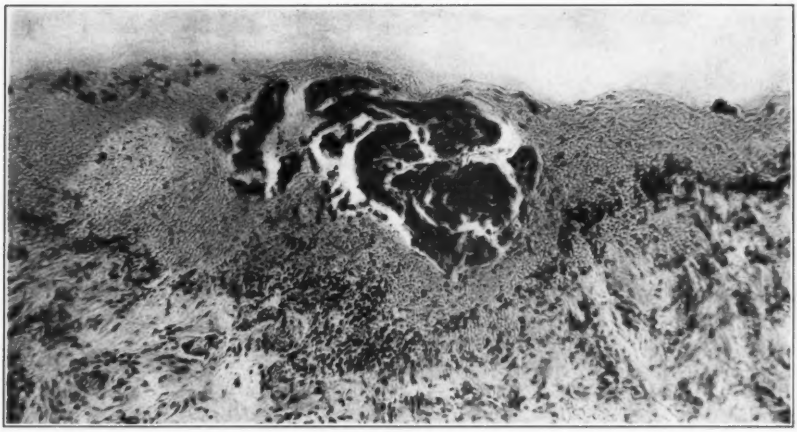


13

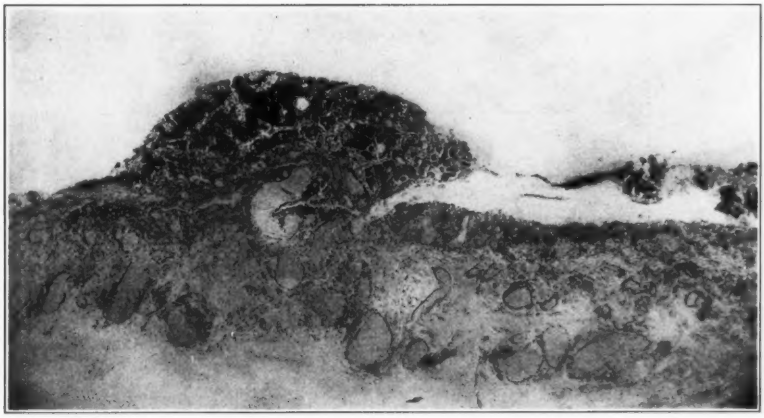
FIG. 14. Photomicrograph of a section through the posterior layer of the mesosalpinx (Case 4). Serial sections were made of the portions of the blocks shown in Figs. 11, 12, 13 and 14. The cancer in each instance was isolated and the slide photographed presented the greatest cross-section of the implant. The peritoneal surface here is denuded of mesothelium and covered by fibrin infiltrated with invading cells, a condition most favorable for the anchoring and encapsulation of a foreign body, the taking of a skin graft, or the implantation of cancer. Enmeshed in this fibrin is a relatively large mass of epithelium-like cells identical in structure with the malignant growth of the ovary (Fig. 10). Note that the activity of the fibroblasts is most marked just beneath the implant where the stimulation would likely be the greatest. × 130.

FIG. 15. Photomicrograph of a later stage in the life history of cancer, anchored to the peritoneum by fibrin similar to the one shown in the preceding illustration. The growth is larger, the scaffold and temporary framework of fibrin has disappeared. Stroma has arisen from the fibroblastic invasion of the temporary framework. A like invasion by vascular endothelium has supplied blood vessels, thus forming granulation tissue. This represents the organizing stage in the development of an implant of polypoid type similar to a like stage in the repair of a wound containing a foreign body. × 25.

FIG. 16. Photomicrograph of a section of the parietal peritoneum with two implants (Case 4). The larger one represents a later (the organized or healed) stage of the condition shown in the preceding illustration. The marked injection of the stroma of the implant and of the peritoneum shown in Fig. 15 has subsided. This implant is more definitely circumscribed and has settled into the underlying peritoneum which has been invaded by the cells of the implant, just as similar cells of a primary cancer invade underlying structures. The smaller implant (to the left) possibly might have arisen from conditions similar to those shown in Figs. 12 and 13. It is evident that the form of the implant varies with its age, the condition of the field of implantation when the cancer cells became anchored, the reaction of the tissues of its host and the rate of growth of the cancer. × 25.



14



15

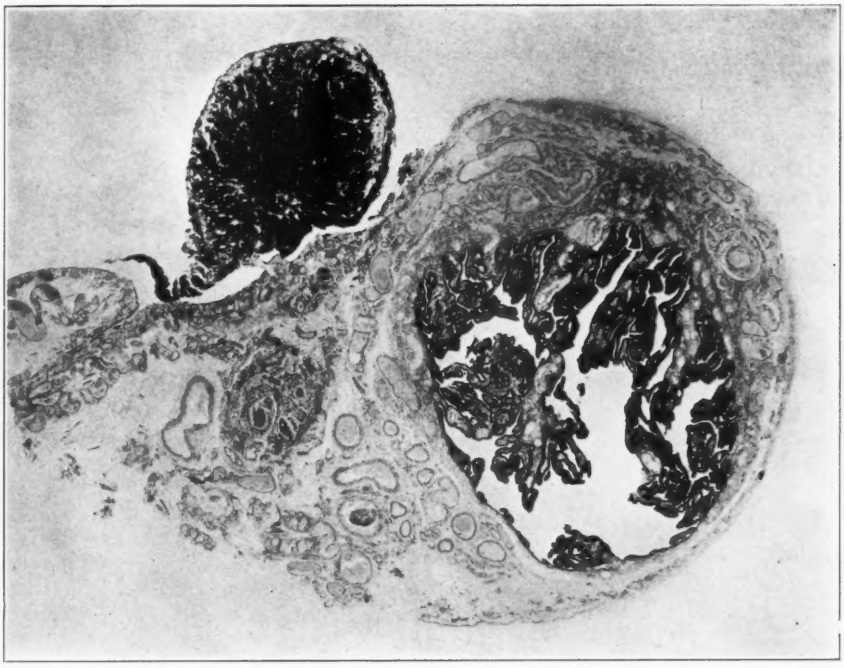
16

Sampson Implantation Peritoneal Carcinomatosis

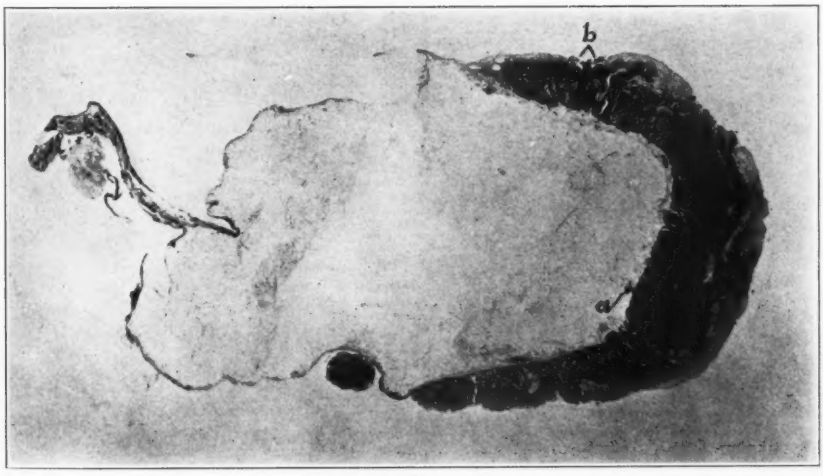
PLATE 87

FIG. 17. Photomicrograph of a cross-section of the Fallopian tube and a portion of its mesentery (Case 4). A polypoid implant is attached to the posterior surface of the mesosalpinx by a single slender vascular pedicle. The development of this type of implant is shown in Figs. 36 and 37. A tail-like process extends from the polypoid implant. It appears to be an outgrowth from the latter, but could well have arisen from the organization of a thin sheet of fibrin with enmeshed cancer cells. It is often of great importance to make serial sections of interesting lesions. Otherwise a single cross-section of a tail-like process similar to the one shown above may appear to be a small implant. This tail-like process was broad and flat, similar to a beaver's tail and not like that of a mouse as it appears in the photomicrograph. × 10.

FIG. 18. Photomicrograph of a longitudinal section of an epiploical appendage (Case 4). The appendage is capped by cancer. The condition shown here well demonstrates how cancer, once implanted on the peritoneum, spreads over the surface of the latter, invades its underlying tissue (a) and when it pierces its capsule (b) then cancer cells might escape into the peritoneal cavity and be a source of cancer cells in the ascitic fluid; these in turn might become implanted on the peritoneum, causing secondary implants. The pedicle-like structure to the left arose from crushing the base of the appendage with a clamp when it was removed. Cancer was not found in the lymphatics of the appendage. × 10.



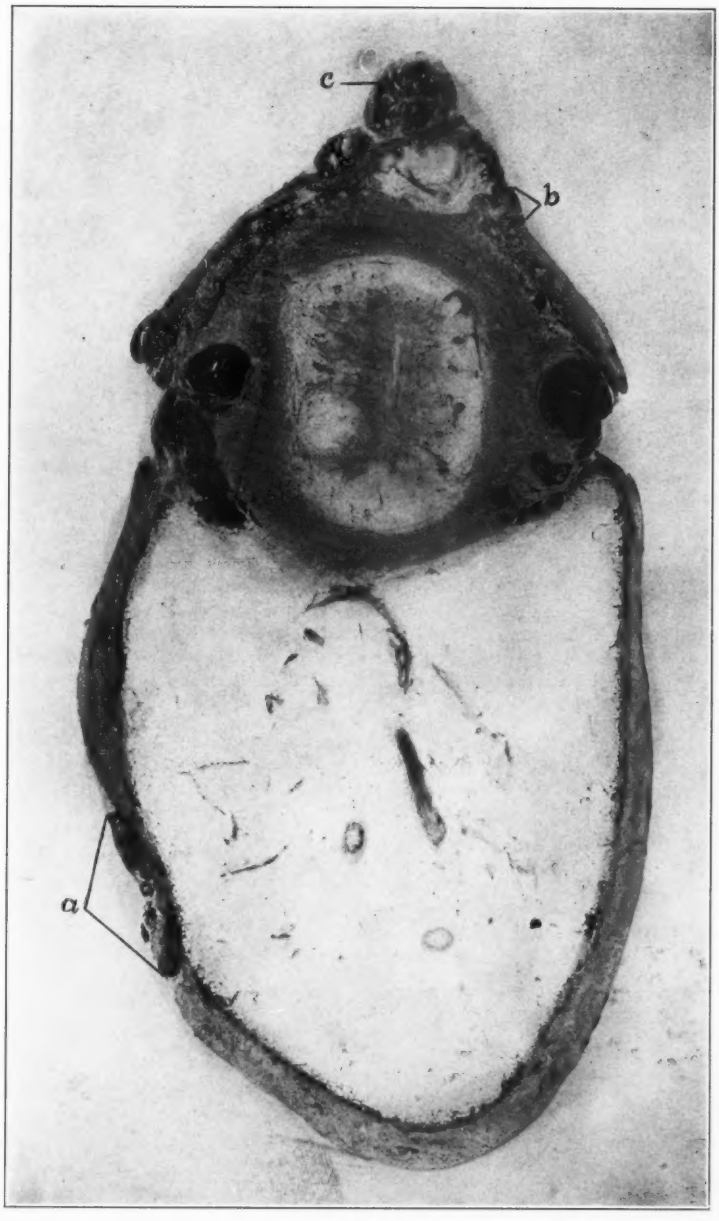
17



18

PLATE 88

FIG. 19. Photomicrograph of a cross-section of an obliterated appendix with a portion of its mesentery from a patient with peritoneal carcinomatosis associated with bilateral ovarian cancer (Case 5). Many interesting peritoneal lesions are present in this section. The wall of the appendix has been invaded on both sides at the attachment of its mesentery. This is a frequent site for implants on the intestinal tract. These two lesions are apparently of the same age, and as in many primary cancers, the oldest portion of the growth has sloughed, leaving a punched-out ulcer. An assumed earlier implant is present on the peritoneum of the mesentery ((a) see also Fig. 22), and a still earlier one on the peritoneum of the appendix ((b) see also Fig. 21). An encapsulated implant (c) is present at the apex of the illustration. Many of the cancer cells in this implant are dead. Cancer was not present in the lymphatics of the meso-appendix. For a probable source of the peritoneal lesions shown in this illustration see Fig. 20. × 10.



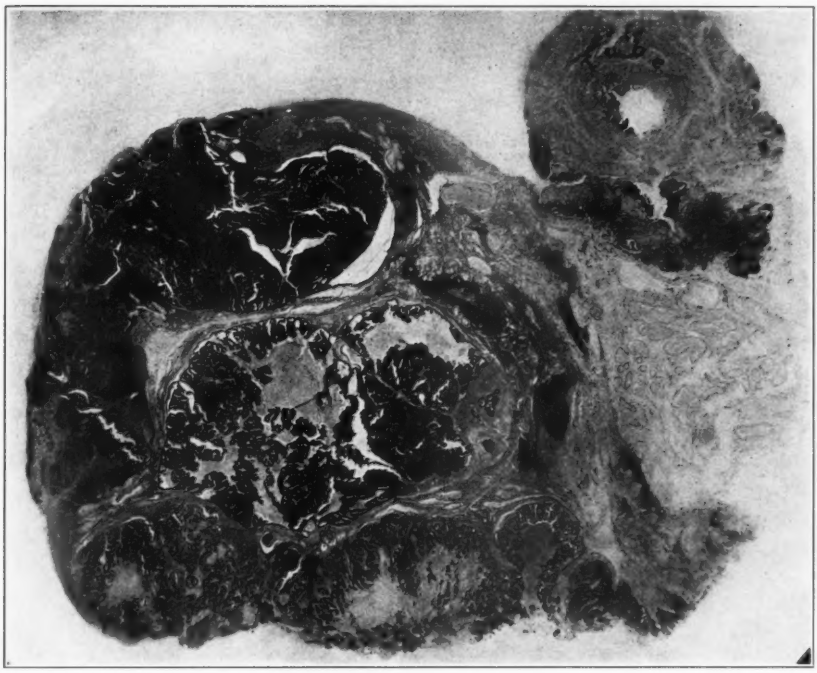
19

PLATE 89

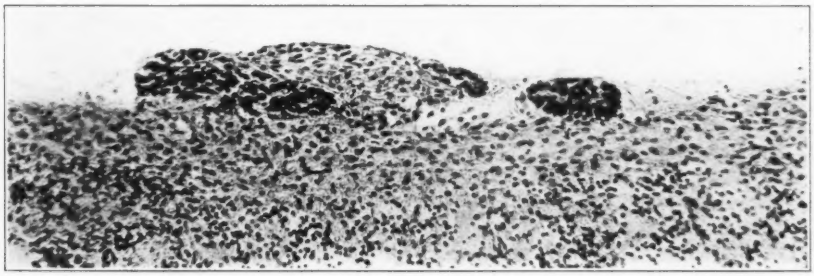
FIG. 20. Photomicrograph of a cross-section of the ovary with the tube and a portion of the meso-ovarium and mesosalpinx attached (Case 5). Almost the entire ovary has been replaced by cancer. The ovary was adherent by its lateral surface (lower border of the illustration) to the posterior layer of the broad ligament, invading the latter by direct extension. In other situations the cancer has extended through the capsule of the ovary, thus readily permitting the dissemination of cancer cells into the peritoneal cavity. The posterior layer of the mesosalpinx is invaded by cancer from its peritoneal surface. Similar but more superficial lesions are present on the anterior surface of the tube. × 5.

FIG. 21. Photomicrograph of apparently an early implantation on the peritoneal surface of the appendix indicated by (b) of Fig. 19. As serial sections were not made of this block, one cannot exclude the probability that the condition shown here represents a section through the advancing edge of a larger implant over the surface of the peritoneum, rather than an early implantation. The peritoneum shows the characteristic lesion of chronic irritation; the hyperchromatic cancer cells are readily discernible. In one situation, to the right, they are surrounded by fibrin, but to the left they are partially surrounded by cells which I believe may be of mesothelial origin. I doubt if cancer cells can become implanted on intact mesothelium, but once established they might extend over the surface of the same or replace it. × 130.

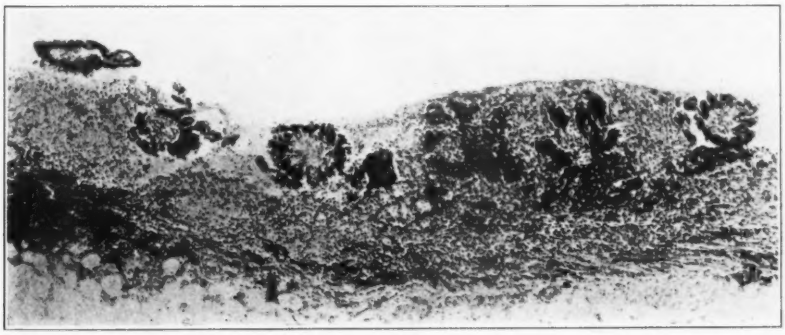
FIG. 22. Photomicrograph of the cancer involving the peritoneum of the mesoappendix, indicated by (a) of Fig. 19. Cancer embedded in the thickened peritoneum is shown at the right, and at the left, cancer enmeshed in fibrin infiltrated with cells growing out from the injured peritoneum. Serial sections were not made, and for this reason the exact relation between the cancer in the apparently different stages of its encapsulation cannot be determined. The cancer at the left could have arisen from the direct extension of that at the right rather than from a fresh implantation from the ovarian tumor. × 60.



20



21



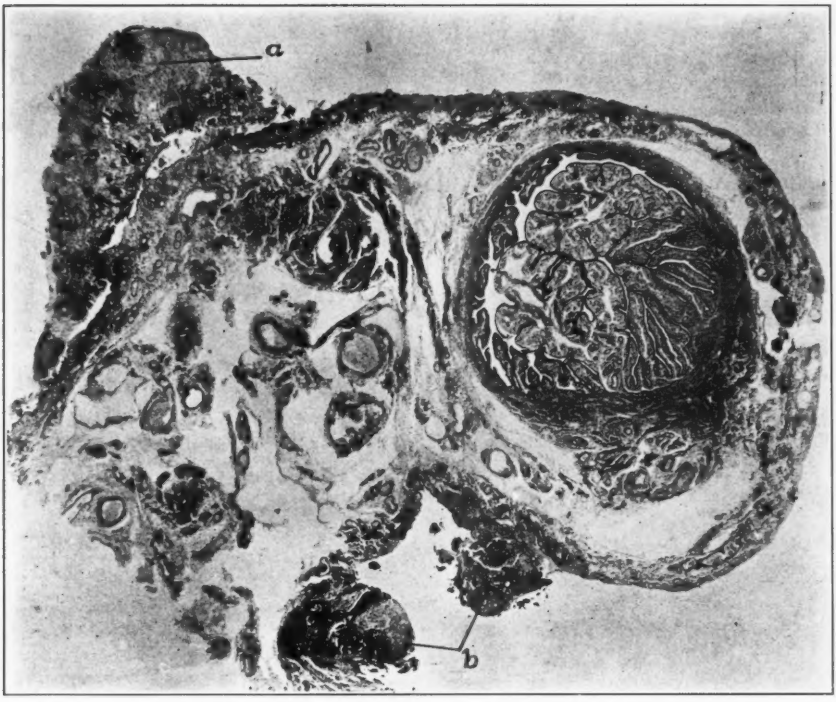
22

Sampson Implantation Peritoneal Carcinomatosis

PLATE 90

FIG. 23. Photomicrograph of a cross-section of the tube with a portion of its mesentery (Case 5). Cancer was found only on the surface of the peritoneum: (a) is a large sessile, polypoid implant (granulation tissue stage) with a fenestrated base. Multiple pedicles have arisen from the organization of fibrin deposited on the surface of the peritoneum in an exudate which had poured out through multiple breaks in the mesothelial covering of the injured peritoneum. Through these same breaks fibroblasts and vascular endothelium grew out invading the fibrin. The fenestra are but the spaces between the pedicles. On the opposite side of the mesosalpinx are two older (organized) polypoid implants (b) representing the scar tissue stage in the healing of wounds caused by foreign bodies lodging on the peritoneum. × 10.

FIG. 24. Photomicrograph of a longitudinal section of an epiploical appendage (Case 5) showing various types of peritoneal implants, and various stages in their life history. At (a) the cancer is embedded in the thickened peritoneum like a foreign body. At (b) the implant is similar to the one shown at (a), but like a primary growth it has invaded the deeper tissues of the appendage (see Fig. 26). Implant (c) represents either an older implant or one more advanced than that shown at (a). It has invaded the tissues of the appendage en masse, as a primary cancer often does, and like the latter necrosis with sloughing and ulcer formation has occurred in the oldest part of the tumor. At (d) a papillary implant without encapsulation is spreading over the surface of the appendage (see Fig. 27). Cancer was not found in the lymphatics of the appendage. × 10.



23

24

Sampson Implantation Peritoneal Carcinomatosis

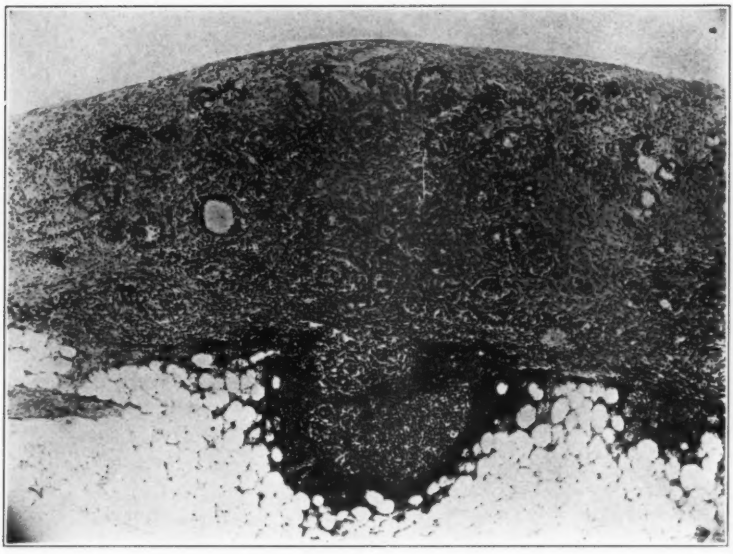
PLATE 91

FIG. 25. Photomicrograph of a portion of the peritoneum to the right of implant (c), Fig. 24. It well demonstrates the way newly formed tissue grows over cancer cells, later causing the condition shown in the next illustration. I am unable to state whether the clumps of cancer cells are a recent implantation, or a direct extension from the older implant. Serial sections were not made of this block. × 60.

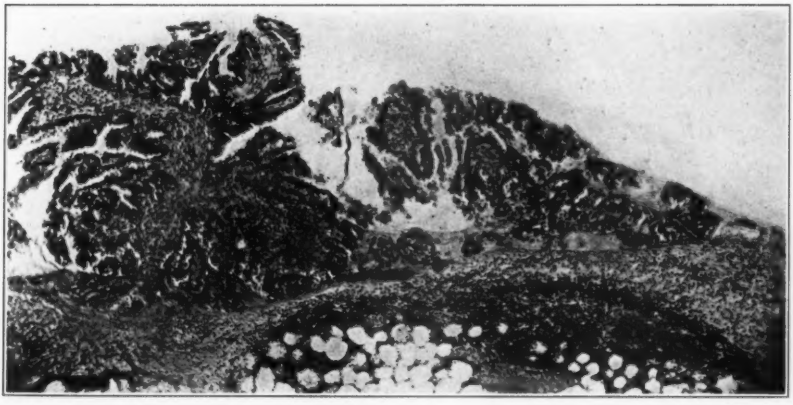
FIG. 26. Photomicrograph of a portion of implant (b), Fig. 24. The implant is fully organized (the wound has been healed), but the cancer has invaded the subperitoneal tissues of the appendage like a primary tumor. Note the reaction to this invasion. × 60.

FIG. 27. Photomicrograph of a portion of implant (d), Fig. 24. It is a papillary tumor presenting the appearance of a primary cancer arising from the mesothelium of the peritoneum. I believe that it is an implant of the engrafted type without encapsulation, similar to the taking of a skin graft. Cancer cells were probably anchored to the peritoneum by fibrin and the fibrin covering the anchored cells never became organized, or if it did it was later destroyed by the cancer. Note the way the growth spreads over the surface of the appendage (to the right). Cells could escape from such a tumor into the peritoneal cavity as easily as from the primary tumor and might cause secondary peritoneal implantations. × 60.

25



26



27

Sampson Implantation Peritoneal Carcinomatosis

PLATE 92

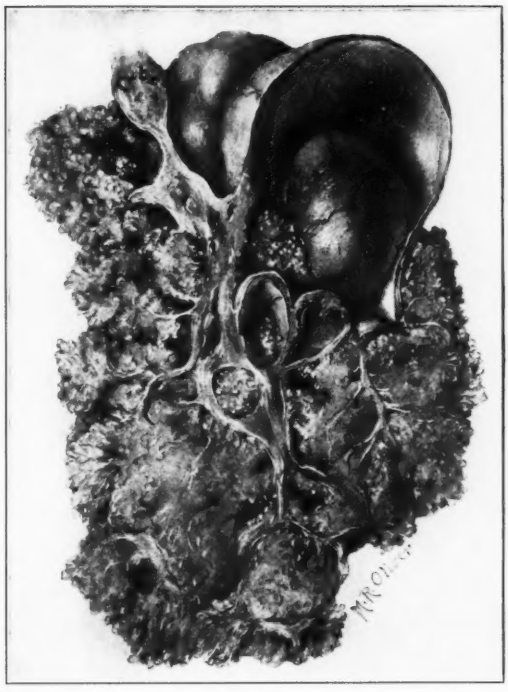
FIG. 28. Cross-section of an ovary and tube from a patient with bilateral ovarian carcinoma, associated with ascites and a marked peritoneal reaction. This reaction manifested itself by an extensive distribution of granulation tissue on the surface of the peritoneum throughout the lower peritoneal cavity (Case 6). Cancer was not found in the lymphatics in any of the sections from this case. (Natural size.)

FIG. 29. Photomicrograph of a section through the exposed surface of the tumor shown in the preceding illustration. It is a papillary adenocarcinoma. A study of the conditions shown in this and the preceding illustration should impress one with two interesting probabilities — one, that cancer cells might easily escape from the surface of such a growth, and the other, that secretions, from the exposed cancer, escaping into the peritoneal cavity, as well as the cancer cells, possibly present in that fluid, might irritate (injure) the peritoneum. × 60.

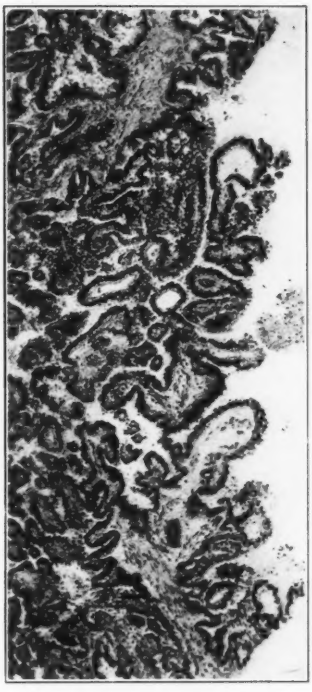
FIG. 30. Photomicrograph of a section of granulation tissue on the surface of an epiploical appendage (Case 6); see also Fig. 33. A clump of epithelium-like cells (c) is attached to the surface of a strand of fibrin. These cells both in appearance and arrangement are similar to those of the primary tumor shown in Fig. 29. × 60.

FIG. 31. Photomicrograph of a section of granulation tissue on the surface of the appendix (Case 6). Epithelium-like cells (c) with an appearance and arrangement identical with similar cells in the two preceding illustrations are enmeshed in the granulation tissue. It might easily represent a later stage of that shown in the preceding illustration. × 60.

FIG. 32. Photomicrograph through granulation tissue on the surface of the mesosalpinx (Case 6). Epithelium-like cells with an appearance and arrangement identical with those in the three preceding sections are enmeshed in newly formed granulation tissue. On account of the proximity of the mesosalpinx to the primary tumor, these cells might have arisen from a direct extension of the primary growth, but this does not hold true for similar conditions in granulation tissue on the epiploical appendage and the appendix. × 60.



28



29

30

31

32

Sampson

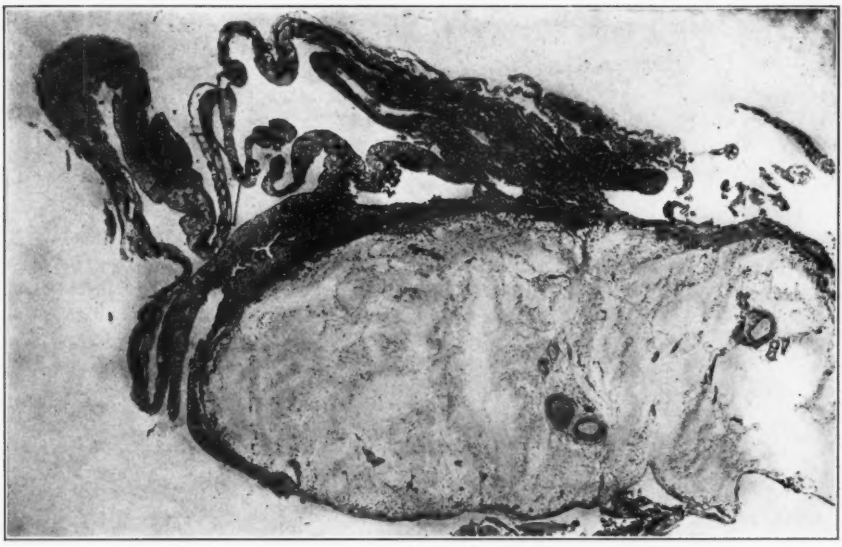
Implantation Peritoneal Carcinomatosis

PLATE 93

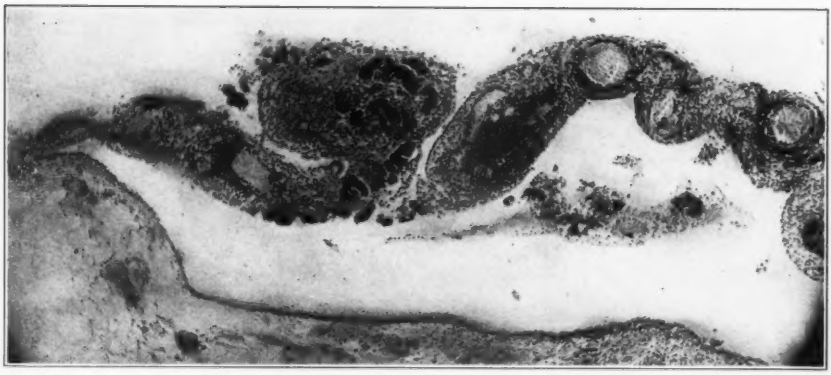
FIG. 33. Photomicrograph of a cross-section of an epiploical appendage with granulation tissue on its peritoneal surface and mainly on one side of the appendage (Case 6). Note that the peritoneum covered with granulation tissue is much thicker than that without it. This suggests that the irritant causing the injury must have been applied more intimately to some portions of the peritoneum of the appendage than to others. As a result of the injury an exudate must have poured into the peritoneal cavity through breaks in the mesothelial covering of the appendage, leading to a deposit of fibrin on its surface. Through these same breaks in the covering of the appendage fibroblasts and vascular endothelium grew out, invaded the fibrin already described, and caused the granulation tissue shown above. The primary ovarian tumor (Figs. 28 and 29) suggests a source of the peritoneal irritant and the presence of cancer cells in this granulation tissue (see Fig. 30) taken at (c). Fig. 33 suggests that they are the irritant. × 10.

FIG. 34. Photomicrograph of granulation tissue on the peritoneal surface of an epiploical appendage from a patient with peritoneal carcinomatosis, associated with a malignant cyst of the left ovary. Clumps of cancer cells are enmeshed in the granulation tissue and also in fibrin just beneath it. The condition shown here could represent a little later stage of that shown in the preceding illustration, namely the organization of fibrin arising on the surface of the peritoneum as a reaction to some irritant which had escaped into the peritoneal cavity and was held captive in the organizing fibrin. × 25.

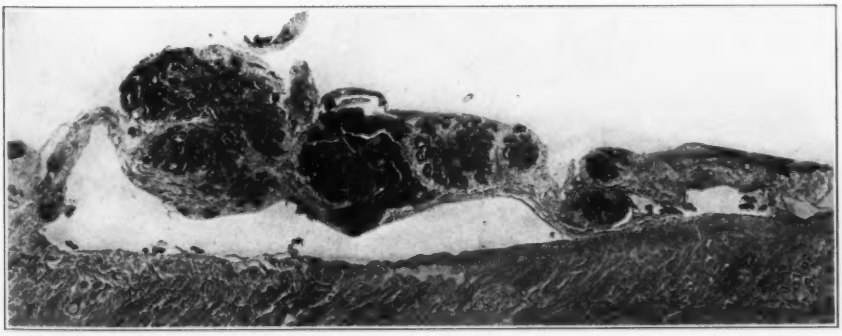
FIG. 35. Photomicrograph of a portion of the posterior wall of the uterus with fully organized adhesions on its surface in which cancer is embedded, from a patient with an extensive peritoneal carcinomatosis associated with a malignant cyst of the left ovary. The condition shown here represents a later stage of the one shown in the preceding illustration. The adhesions are fully organized, similar to the healed or scar tissue stage in the healing of a wound of the peritoneum caused by foreign bodies. Compare with Fig. 34 and note the marked growth of the cancer in the adhesions. The acute reaction of the former lesion has subsided, but the adhesions persist and the cancer continues to grow. Clumps of cancer cells are enmeshed in fibrin beneath the adhesions (to the left) probably representing a recent fixation of these cells on the peritoneum. × 10.



33



34



35

Sampson Implantation Peritoneal Carcinomatosis

PLATE 94

FIG. 36. Photomicrograph of a longitudinal section of a polypoid implant which was attached to the posterior layer of the broad ligament by a slender vascular pedicle. The patient had peritoneal carcinomatosis associated with bilateral ovarian cancer. This represents the organizing or granulation tissue stage in the life history of this type of implant. The granulation tissue appears to have wrapped itself about the clumps of cancer cells. Probably the cancer cells were first caught in fibrin dangling into the peritoneal cavity, and this was invaded by fibroblasts and vascular endothelium, forming granulation tissue. × 25.

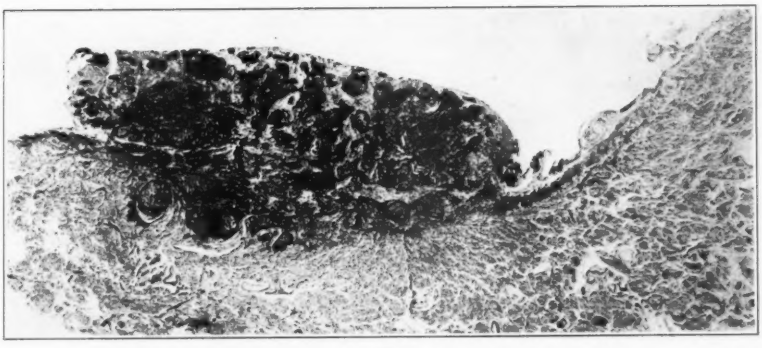
FIG. 37. Photomicrograph of a longitudinal section of another polypoid implant with a slender pedicle, from the same patient as the one shown in the preceding illustration. The pedicle of this implant was attached to adhesions between the tube and ovary. It is a fully organized implant and may have resulted from a condition similar to the one shown in the preceding illustration. Compare with the condition shown in Fig. 36 and note the evident growth of the cancer. × 25.

FIG. 38. Photomicrograph of a sessile polypoid implant on the posterior surface of the uterus. Like a primary growth it has invaded the uterine wall. It is from the same patient as the ones shown in the two preceding illustrations. × 25.

FIG. 39. Photomicrograph of two implants on the posterior surface of the uterus. Judging by the increased amount of scar tissue, they are older implants than the one shown in Fig. 38. All are from the same patient. × 10.

36

37



38

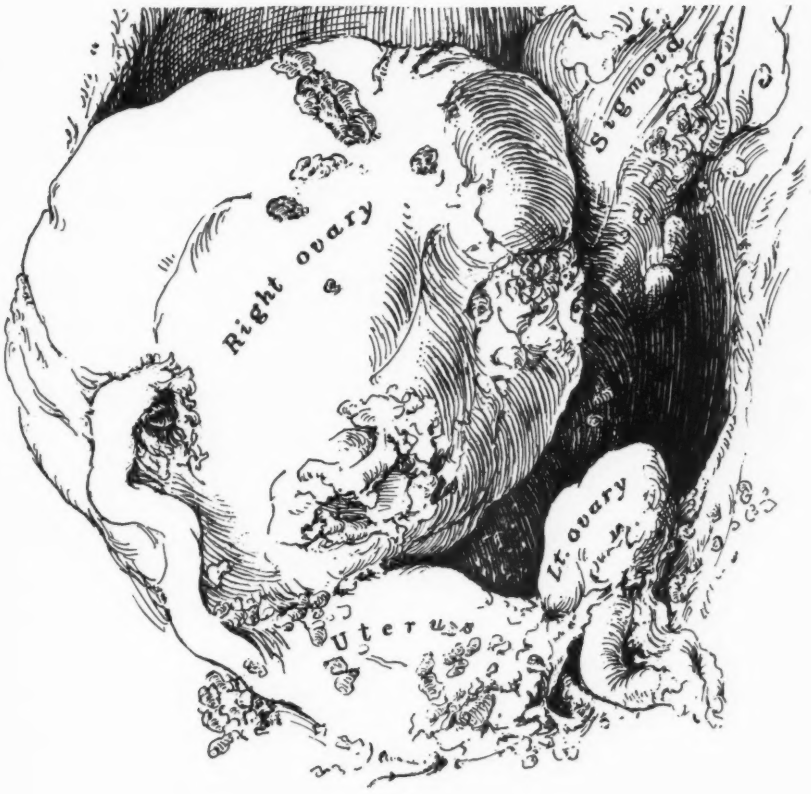
39

Sampson Implantation Peritoneal Carcinomatosis

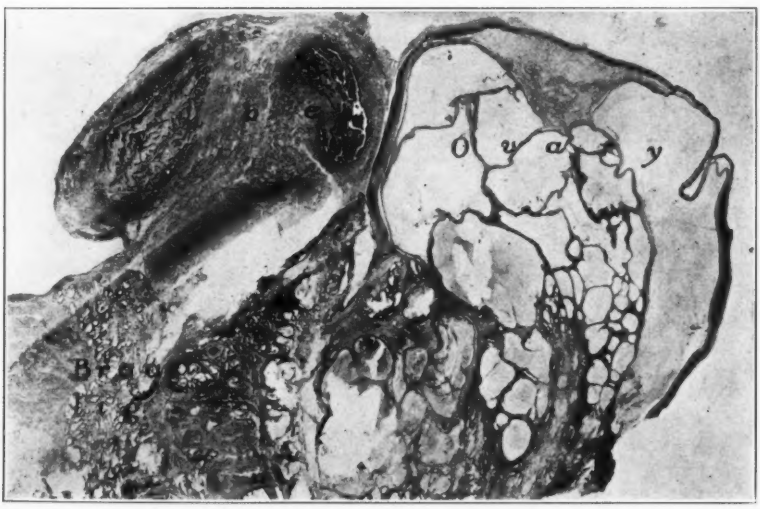
PLATE 95

FIG. 40. Pelvic contents from a patient with a peritoneal carcinomatosis associated with a cystic adenocarcinoma of the right ovary (Case 7). The peritoneal carcinomatosis was general, with the greatest involvement in the posterior cul-de-sac and omentum. In places, the cancer had extended through the capsule of the ovary and was thus exposed to the peritoneal cavity. (Natural size.)

FIG. 41. Photomicrograph of a cross-section of the left ovary and tube and portion of the broad ligament (Case 7). Note that the latter is infiltrated with cancer and the ovary has been entirely replaced by the growth, identical in structure with that of the right ovary. The tube is free of cancer. The cancer in the left ovary was probably secondary to that in the right ovary. It had apparently invaded the ovary through its hilum. Is it from metastasis through the lymphatics or blood stream? Implantations on the posterior surface of the broad ligament, with invasion of the latter, and thence direct extension through the hilum of the ovary, could easily account for the condition shown in this photomicrograph. Cancer was not found in any of the retroperitoneal or mediastinal lymph nodes. × 3.



40



41

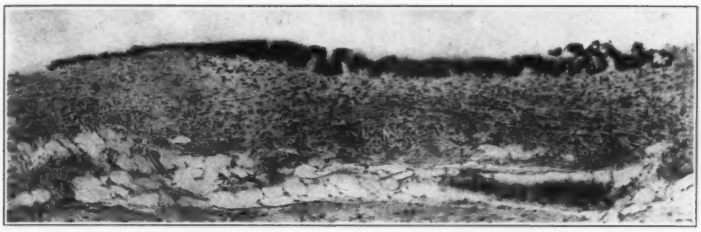
PLATE 96

FIG. 42. Photomicrograph of an apparent metastasis on the under surface of the diaphragm (Case 7). Is it a true metastatic growth or did it arise from malignant changes in the mesothelium? I believe that it is probably an implantation of the skin graft type. The cancer cells became implanted without encapsulation. They were probably anchored with fibrin, but the latter was never organized by fibroblasts. × 60.

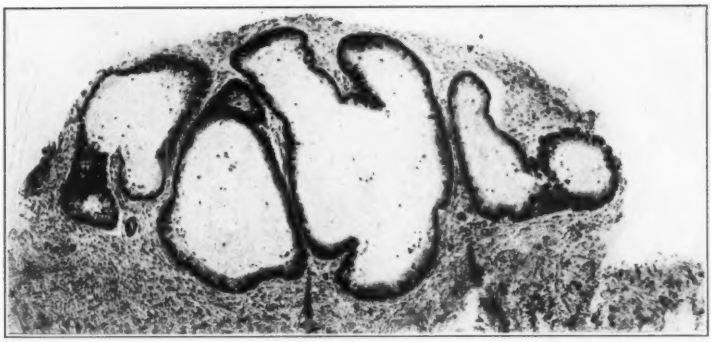
FIG. 43. Photomicrograph of an encapsulated implant on the diaphragm (Case 7). Cancer cells, in ascitic fluid (there was a large amount of it in this case), could easily reach the under surface of diaphragm on which the metastases shown in Figs. 42, 43, 44 and 45 were situated. × 60.

FIG. 44. Photomicrograph of another implant on the diaphragm. It is apparently an older implant than the preceding one. It is broader, flatter and there is more connective tissue. × 60.

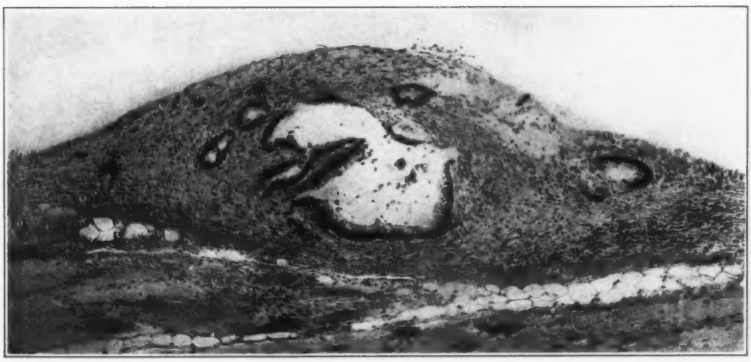
FIG. 45. Photomicrograph of still another implant on the diaphragm, which I believe is older than the preceding one. It is flatter, larger and there is a deeper invasion of the underlying tissues and a more extensive invasion of its capsule. Cancer is present on the surface of the implant at (a). Does it represent a fresh implantation of cancer in this situation or an extension of the cancer through the capsule to its surface? Serial sections were not made of this metastasis and therefore this point cannot be determined. × 25.



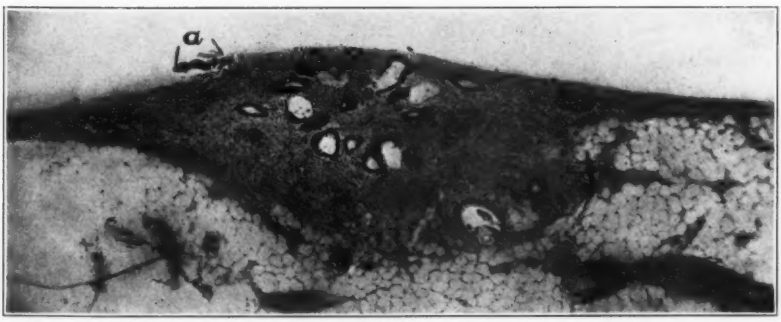
42



43



44



45

PLATE 97

FIG. 46. Photomicrograph of a section of a portion of an epiploical appendage showing two implants of papillary type — the larger one is partially encapsulated — from a patient with extensive peritoneal carcinomatosis associated with bilateral ovarian cancer. An encapsulated implant from the same patient is shown in Fig. 48. × 25.

Fig. 47. Photomicrograph of a section of a portion of an epiploical appendage from a patient with a condition similar to the preceding one. The patients were sisters. Note the similarity in the two tumors. The tumors shown in Figs. 46 and 47 have the appearance of primary growths arising from the mesothelium of the appendage. I believe that they are metastatic by implantation from the ovarian tumors or from other implants. Implantation may have occurred without encapsulation, just as the epithelium of a Thiersch skin graft takes. Even if primarily covered by fibrin this may not have become organized and therefore disappeared. It is also possible that encapsulated cancer may destroy the capsule over it. A study of the condition shown in these two photomicrographs emphasizes the ease with which cancer cells could escape from these tumors into the peritoneal cavity and give rise to secondary implants. × 25.

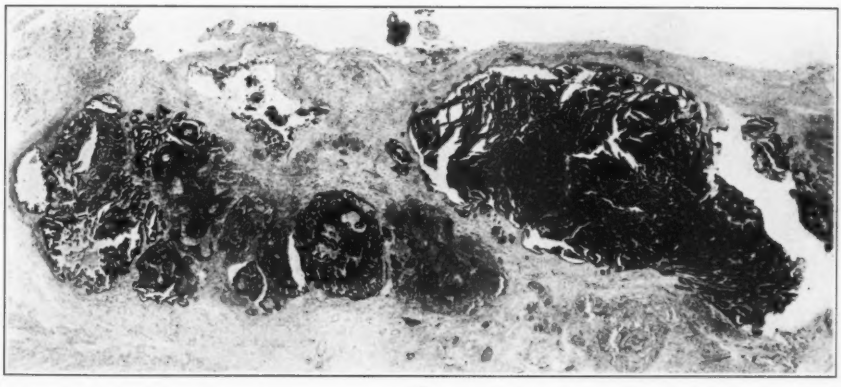
FIG. 48. Photomicrograph of cancer embedded in the peritoneum of the anterior cul-de-sac near the uterus, from the same case as the epiploical appendage shown in Fig. 46. I believe that the condition shown here may have arisen from implantation on the peritoneum with encapsulation and subsequent growth of the cancer, rather than from cancer cells carried through the lymph or blood vessels. × 10.

FIG. 49. Photomicrograph of a section of sediment of ascitic fluid obtained from the same patient as the implant shown in Fig. 47, six months after a previous operation. The mass of pale cells I believe is of mesothelial origin. The clumps of hyperchromatic cells with a glandular arrangement could well be cancer cells which had escaped into the peritoneal cavity from implants such as the one shown in Fig. 47, or they may have been present in the fluid at the first operation when the uterus, tubes and ovaries were removed. × 60.

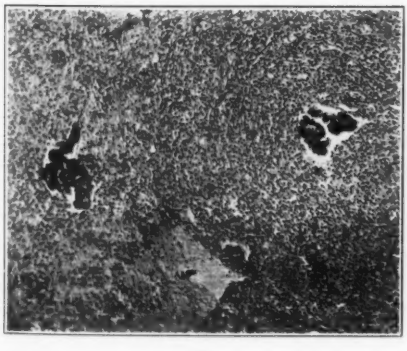
FIG. 50. Photomicrograph of a small peritoneal implant removed at the second operation. It is evidently an old implant probably present at the first operation. × 60.

46

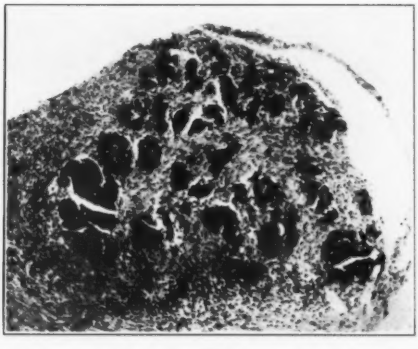
47



48



49



50

Sampson

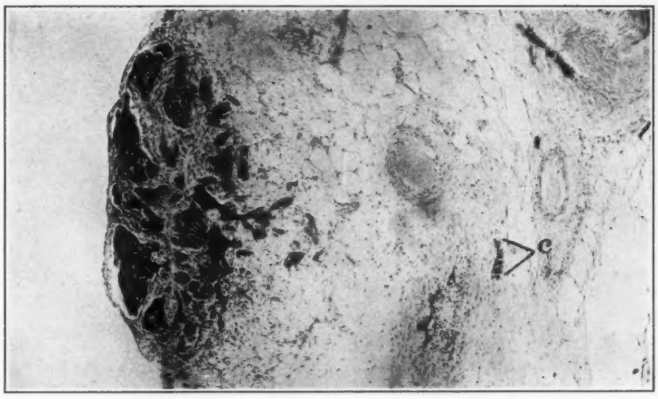
Implantation Peritoneal Carcinomatosis

Plate 98

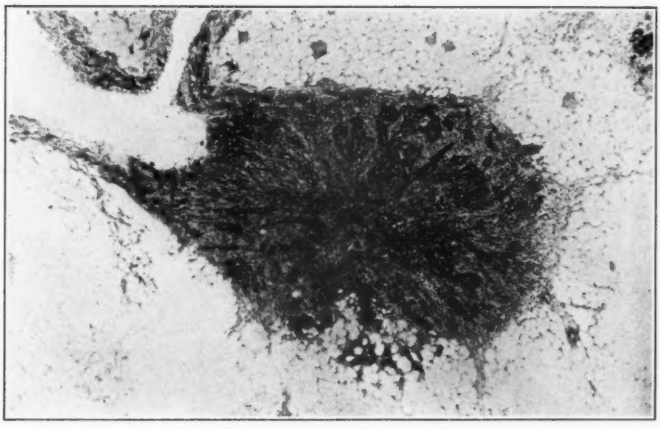
Fig. 51. Photomicrograph of a small implant on the parietal peritoneum (Case 4). Compare with Fig. 16 from the same patient. It appears older than the larger one shown in Fig. 16. It is flatter, more definitely encapsulated, and is more deeply sunken in the peritoneal tissues. Fine root-like processes of cancer from the base of the implant have invaded the deeper tissues of the peritoneum. Cancer is present in a lymphatic at (c). Every metastatic tumor has the same potentialities of invasion and dissemination as a primary tumor in a similar situation. Some may claim that the cancer in a lymphatic indicates that these are the channels by which cancer cells had escaped from the primary tumor to this situation. × 25.

Fig. 52. Photomicrograph of an implant in an epiploical appendage (Case 2, see also Figs. 3 and 4). Note the invasion of the epiploical appendage and the reaction of the tissues of the appendage to this invasion. It is possible that in time the entire metastasis might have been fully embedded in the appendage. Had the section been at right angles to the one shown here, the cancer might have appeared to be in the center of the appendage. × 25.

Fig. 53. Photomicrograph of a cross-section of an epiploical appendage from a patient with extensive peritoneal carcinomatosis associated with bilateral ovarian carcinoma. I believe that the deeper tissues of the appendage could well have been invaded by cancer implanted on its peritoneal surface. Others might claim that the peritoneal involvement arose from lymphatic metastases from the primary tumor, and the cancer in the deeper tissues of the appendage are an indication of this. × 10.



51



52

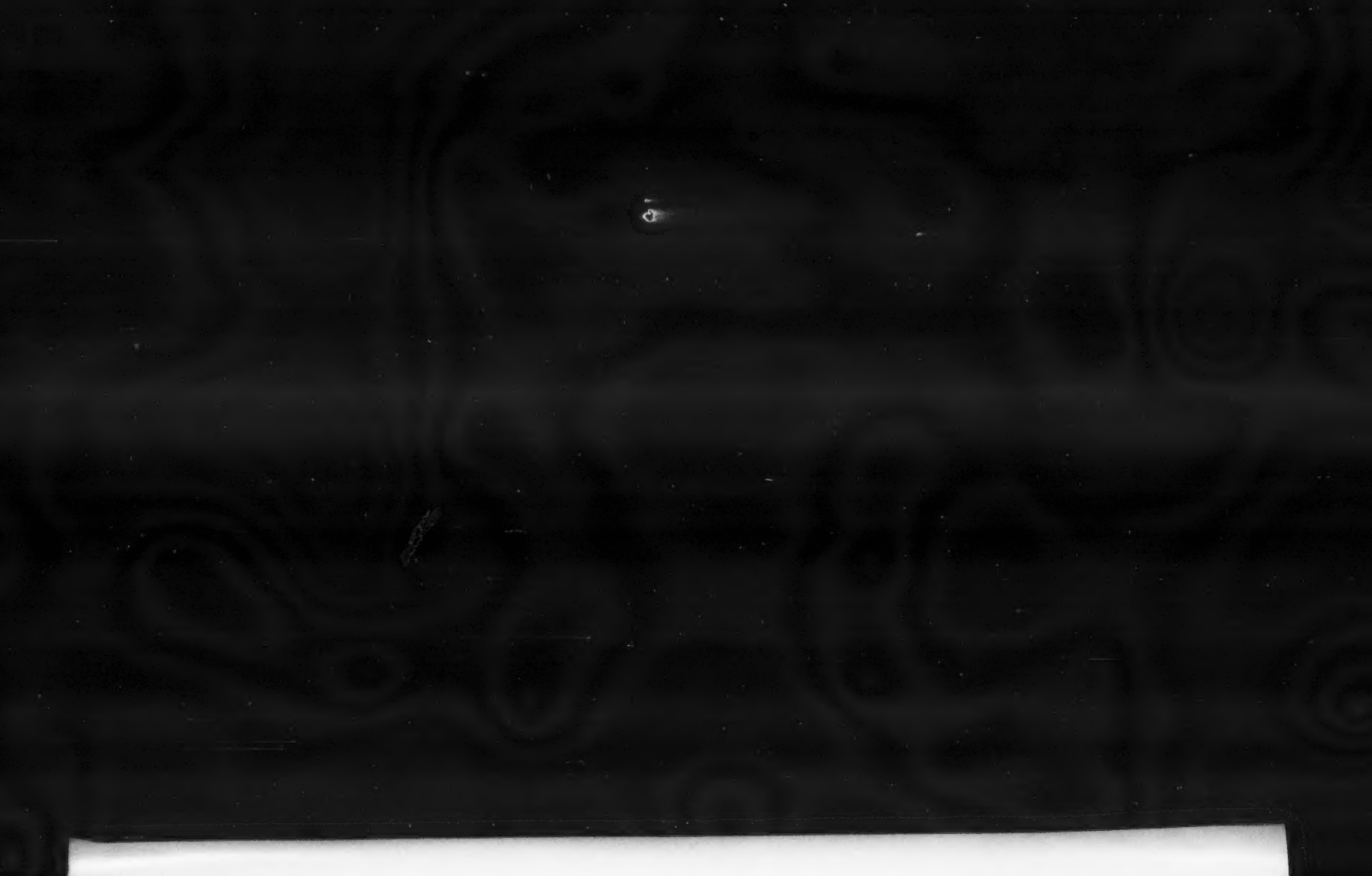
53

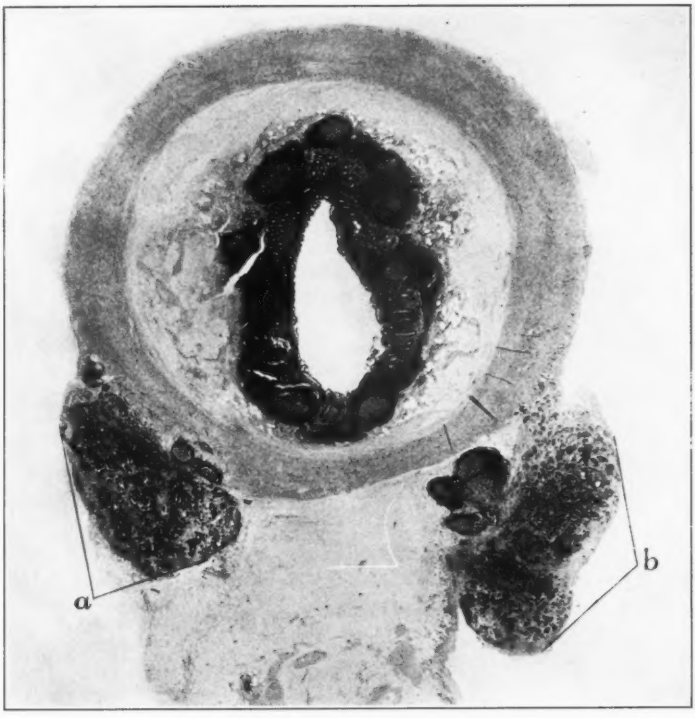
Sampson Implantation Peritoneal Carcinomatosis

PLATE 99

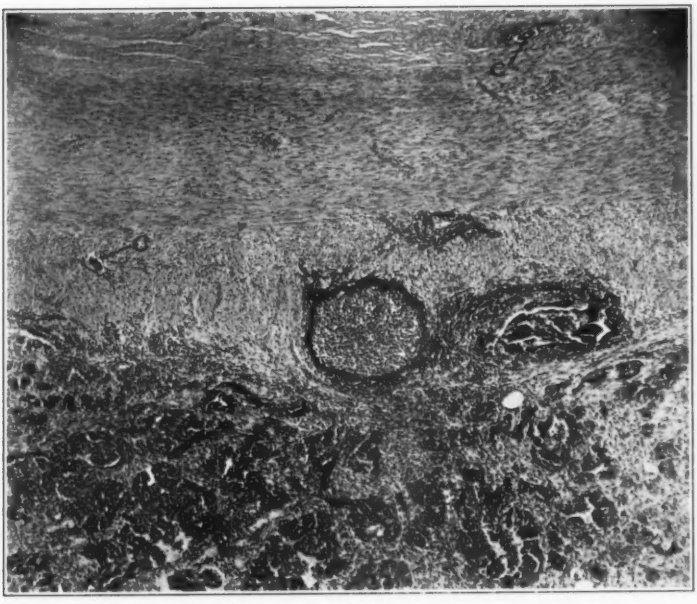
FIG. 54. Photomicrograph of a cross-section of an appendix with a portion of its mesentery, from a patient with extensive peritoneal carcinomatosis associated with bilateral carcinomatous ovarian cysts. Two fully organized implants are shown, one on each side of the appendix at its mesenteric attachment, a frequent site for implantations on the intestinal tract. They are apparently of about the same age, at least they represent the same stage in their development. Compare with Fig. 34 in which is shown an implant in adhesions on the epiploical appendage from the same patient, the latter representing the organizing or granulation tissue stage in the development of peritoneal implants. The cancer in both of the implants shown here has invaded the deeper tissues of its host like that of a primary growth. (See next illustration.) × 10.

FIG. 55. Photomicrograph of a portion of implant (a), Fig. 54, and the wall of the appendix. The cancer cells of the implant have invaded the wall of the appendix as those of a primary growth might. Cross-sections of vessels (probably lymphatics) containing cancer cells are shown at (c) and (c). Did the latter arise from the cancer of the implant or from the ovarian tumor? I believe the former. Cancer was not found in the lymph vessels of the meso-appendix. What might be a later stage of the condition shown in these two photomicrographs? × 60.





54



55

PLATE 100

FIG. 56. Photomicrograph of a cross-section of an appendix with a portion of its mesentery, from a patient with peritoneal carcinomatosis associated with a carcinomatous cyst of the left ovary. The peritoneal metastasis (implant) is evidently older than those shown in Fig. 54. It is flatter and more intimately blended with the wall of the appendix and the tissues of its mesentery. The lumen of the appendix is obliterated and the lymphatics of the appendix, as well as those of the mesentery, are permeated with cancer filling them like an injection mass. See Figs. 58 and 59. × 10.

FIG. 57. Photomicrograph showing cancer cells enmeshed in fibrin and partially embedded in granulation tissue from (a) of the preceding illustration. As serial sections were not made of this block it is impossible to state whether this is a recent implantation or a direct extension from the cancer to the left. From a histological standpoint it could be either. × 60.

FIG. 58. Photomicrograph showing the extension of cancer from the base of the implant into the tissues of the wall of the appendix ((b) Fig. 56). Lymph vessels permeated with cancer are shown just beneath this invasion. × 60.

FIG. 59. Photomicrograph showing a portion of the appendix and its mesentery ((c) Fig. 56). There is a gap in the muscularis of the appendix for the entry and exit of vessels. The lymph vessels of both the appendix and its mesentery are filled with cancer cells. It might be claimed that the conditions shown in these photomicrographs indicate that the metastatic cancer of the peritoneum of the appendix came from the ovarian tumor through the lymph vessels. The metastatic growth of the peritoneum presents the histological appearance of an older implant than the one shown in Fig. 54, and therefore with greater opportunities for invasion and lymphatic permeation. I believe it could well represent a later stage of the former. The conditions found in both implants are frequently seen in primary cancers and a metastatic tumor has the same potentialities of invasion and dissemination as a similar primary growth in its situation. × 60.

56 57

58 59

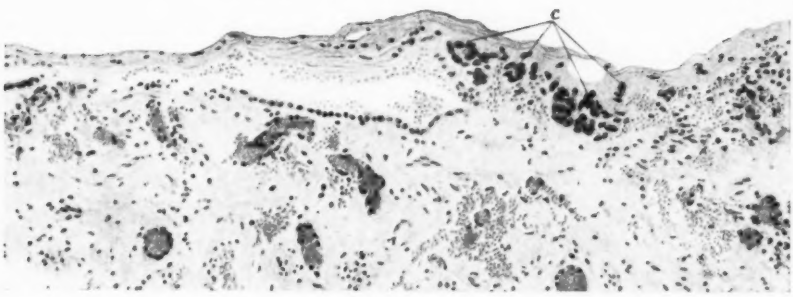
Sampson Implantation Peritoneal Carcinomatosis

Plate 101

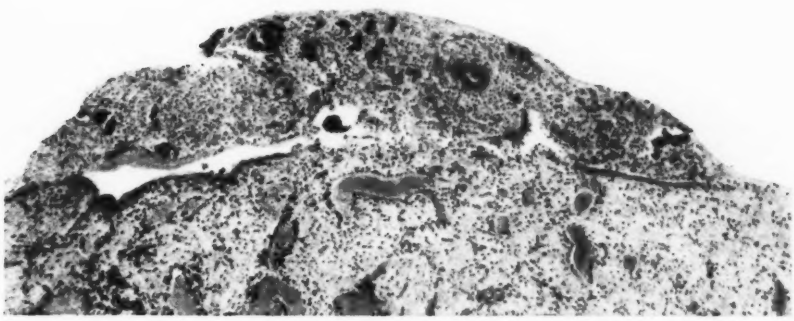
Fig. 60. Colored photomicrograph of the section shown in Fig. 11 (Case 4) to demonstrate better the reaction of the peritoneum to injury caused by cancer cells (c) lodging on its surface. Note the injection of the blood vessels and the other reactions described in Fig. 11. I believe that the condition shown here represents the fixation stage in the life history of the implantation of cancer cells on the peritoneum. × 130.

Fig. 61. Colored photomicrograph of a section of a peritoneal implant on the surface of the Fallopian tube shown in Fig. 20. It represents the organizing stage in the life history of such an implant. The clumps of cancer cells are enmeshed in granulation tissue, a well recognized reaction to peritoneal injury. This undoubtedly arose from the invasion of fibrin, on the surface of the injured peritoneum, by fibroblasts and vascular endothelium growing out through the break in the peritoneal mesothelium shown in the center of the illustration. Compare with Fig. 60 and note the changes in the peritoneum, indicating an irritation of longer duration than that shown in that illustration. × 60.

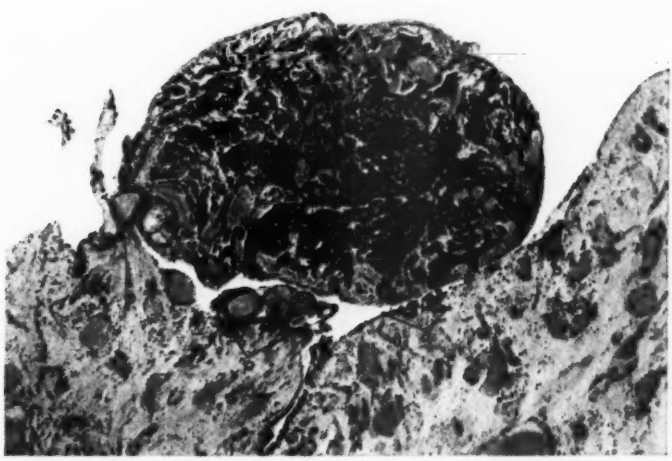
Fig. 62. Colored photomicrograph of a section through an implant on the posterior surface of the mesosalpinx (Case 4). It is a fully organized implant of the polypoid type. Note the evident growth of the cancer in the implant, the attempt at encapsulation, and the extension of the cancer through the capsule in places. An apparent contact peritoneal implant from the larger implant, above it, is situated at (a) and an implant similar to the one shown in Fig. 60 is situated at (b). The latter also might have arisen from cancer cells escaping from the larger implant. The larger implant had two vascular pedicles, portions of which are shown at (p) and (p). × 25.



60



61



62

TOPOGRAPHIC ANATOMY AND HISTOLOGY OF THE VALVES IN THE HUMAN HEART*

LOUIS GROSS, M.D., AND M. A. KUGEL, M.D.†

(From the Laboratories of The Mount Sinai Hospital, New York City)

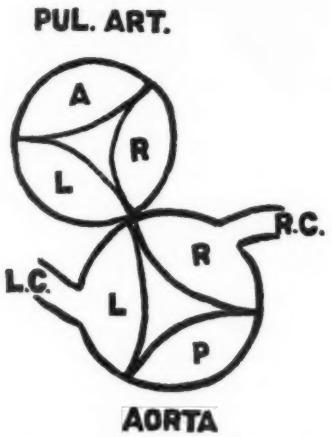
An accurate knowledge of the structure of the valves in the human heart is of prime importance for at least three very practical reasons. The first is that the various anatomical peculiarities in the valve leaflets suggest an explanation for at least some of the mechanical components entering into the localization of inflammatory, as well as of degenerative (atherosclerotic) processes in these sites. Secondly, the insight into pathological processes thus obtained helps one differentiate these lesions from one another. Thirdly, one is better able to cope with the long disputed question as to whether or not blood vessels or myocardium normally exist in valves. This is not a merely academic question, myocardium having been implicated by various authors as the source of the alleged existence of blood vessels in normal valves.

The descriptions of the valves found in the literature are difficult to follow because the best ones are given by workers who limit themselves to only one or two valves and, since the terminology used is often different, confusion results. As an illustration of this, one may mention that the same layer of tissue in the valve is called "Klappenplatte" by Seipp, "Mittelschicht" by Beitzke, "Grundstock" by Königer, "Lamina Fibrosa" by Tandler, "Klappenskelet" by Benninghoff, and by other investigators is referred to by number. Perhaps the greatest source of dispute lies in the fact that, with rare exceptions, no attempt is made to set definite limits to the valve. It may be said at once that this factor alone is largely responsible for the controversy on the extent to which myocardium has been found in valves and, because of this, on the frequency with which blood vessels have been found in the valves.

* This study was aided by a grant from the Lucius N. Littauer Foundation.
Received for publication June 22, 1931.

† Moses Heineman Fellow in Pathology.

In this paper we propose to describe and picture the valves of the human heart, both in their more minute histology as well as in their typographical relations to other structures, setting as far as possible the limit as to what is valve and where the adjoining cardiac structures begin. We shall also attempt to establish a descriptive nomenclature and indicate at the same time what changes occur in the various structures at different age periods. In our experience with pathological anatomy we feel that the latter point, to which practically no attention has been paid, may carry with it some leads on the mechanical factors concerned with valvular disease.



TEXT-FIG. 1. Diagram to illustrate terminology of pulmonary and aortic valve cusps. LC, left coronary artery; RC, right coronary artery; L, left cusp; R, right cusp; A, anterior cusp; P, posterior cusp.

The plan is to discuss the general structure of valves in brief and to follow this by a detailed description of each cusp, instead of grouping together the semilunar valves and the auriculoventricular valves, as has been the general practice heretofore.

In this article the B. N. A. nomenclature is used (Text-Fig. 1). Thus, the aortic cusp corresponding to the ostium of the right coronary artery is called the right cusp, that corresponding to the left coronary ostium is called the left cusp and the non-coronary cusp is called the posterior. The right pulmonary cusp is that one which is apposed to the right aortic cusp. The left pulmonary cusp is that

one which is apposed to the left aortic cusp. The remaining pulmonary cusp is the anterior. The terminology of the auriculoventricular valve cusps needs no special explanation.

Methods and Material

The descriptions which follow are based on an examination of 1000 normal human hearts. These were opened in the customary manner, the chambers washed out and packed, and the hearts fixed in a 10 per cent neutral formaldehyde sodium chloride solution.* Occasionally a formaldehyde solution of Mueller's fluid † was used as a fixative and, if the specimens were very fresh (less than six hours postmortem), Bouin's fluid was employed. After fixation, suitable blocks were cut from the heart and at least two slides were stained from each block, one with hematoxylin and eosin, the other with Weigert's elastic and Van Gieson's connective tissue stains. Latterly, Masson's trichrome stains were used with excellent results.

For the hearts available during the latter part of these studies the standardized method for sections described by Gross, Antopol and Sacks was used in order to obtain comparable statistical material.

General Description of Human Heart Valves

Human heart valves have certain general features in common and yet sufficient individual differences to distinguish them sharply from one another. All the valves carry as their main backbone a dense collagenous layer which we propose to call the *Fibrosa* (Figs. 1 and 6). On its auricular aspect in the auriculoventricular valves (Fig. 8), as well as on its ventricular aspect in the semilunar valves (Fig. 6), the *Fibrosa* shows a looser structure which we propose to call the *Spongiosa*. In the semilunar cusps this looser structure may be so conspicuous as to constitute a sharply defined layer.

Each valve cusp is attached at its base to a more or less dense connective tissue structure called *Annulus Fibrosis*. This *Annulus*

* Solution of formaldehyde, U. S. P., 10 parts; 1 per cent sodium chloride solution, 90 parts. This solution is rendered neutral with a weak alkali.

† The formaldehyde solution of Mueller (Formol–Mueller) is prepared as follows: potassium bichromate, 2 parts by weight; water, 100 parts; solution of formaldehyde, U. S. P., 10 parts. The "solution of formaldehyde" is added just before use.

differs considerably with the various cusps in its extent, distribution and connections. Its topography for the aortic valve has been admirably described by Lewis and Grant. There exists, however, an important strategic site which includes part of the base of the valve as well as the adjacent portion of the *Annulus*. We designate this area the *Ring* (Text-Fig. 2). The exact descriptions of the several valve *Rings* will be given under their appropriate headings. *It is of utmost importance to mention here that the valve "Ring" should be considered to constitute the proximal end of a valve leaflet.* This will be taken up further in greater detail.

The *Rings* of the semilunar valves (Fig. 5 and Text-Fig. 2) generally contain a conspicuous *Spongiosa* referred to by Curtis as "un espace triangulaire . . . occupé par un tissue conjonctif spécial." On the other hand, the auriculoventricular valve *Rings* in this area show only a slightly looser structure of the collagenous *Fibrosa* (Fig. 8). We designate these areas *Ring Spongiosa*.

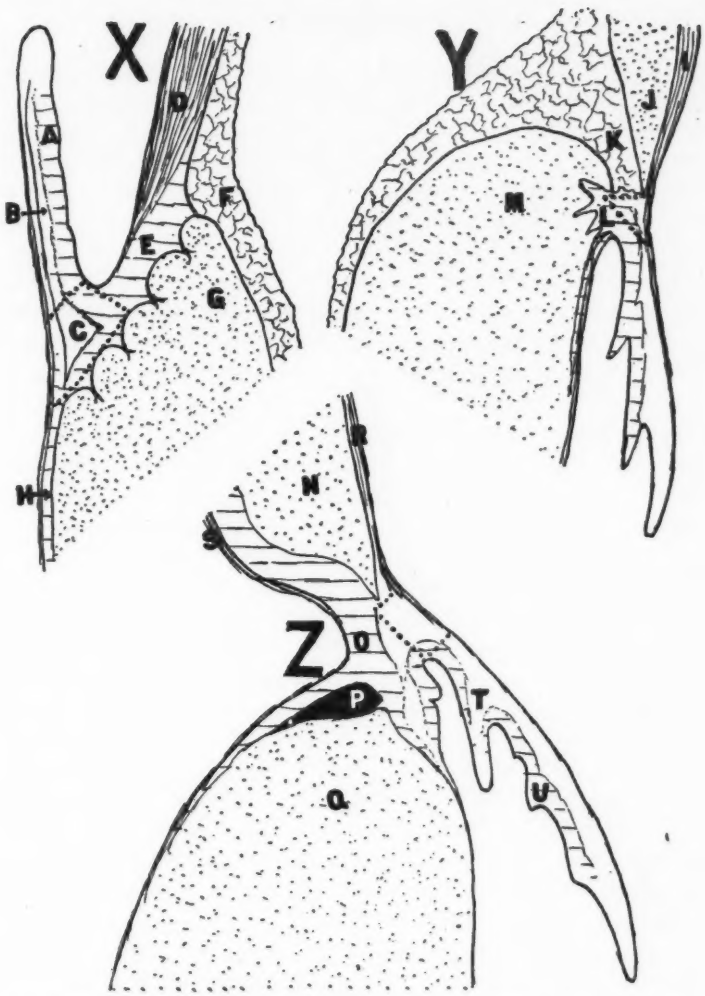
The *Fibrosa*, with its looser layer, is clothed on both sides by a continuation of the arterial intima or ventricular or auricular endocardium, as the case may be. These arterial or endocardial connective tissue mantles contain more or less conspicuous elastic sheets. The elastic sheets which are situated on the outflow surface of the valve (auricular surface of the auriculoventricular valves; ventricular surface of semilunar valves) are generally the heavier and longer (Seipp). Both elastic layers thin out progressively as they approach the tip of the valve.

As a generalization it may be stated that while the separation of these valve layers is already seen in early fetal life, they become more and more clearly defined with advancing postnatal age periods (Veraguth). Furthermore, the differences in their extent, thickness, structure and distribution give to each cusp its individual characteristics.

Aortic Valve

Topography

The topographic relations to their attachments of each aortic cusp, indeed of various parts of each aortic cusp, show wide individual differences. This is due largely to the peculiar linking up of the left and posterior aortic cusps with the anterior leaflet of the mitral valve and the consequent deformation of the auricular myo-



TEXT-FIG. 2. Diagrams of typical semilunar and auriculoventricular valve rings.

X. *Typical Semilunar Cusp.* A, fibrosa; B, valve spongiosa; C, ring spongiosa; D, aorta; E, annulus; F, pericardium; G, ventricular myocardium; H, subvalvular annulus.

Area enclosed by dotted lines is the valve ring.

Y. *Typical Auriculoventricular Cusp.* I, auricular endocardium; J, auricular myocardial wedge; K, pericardial wedge; L, annulus; M, ventricular myocardium.

Area enclosed by dotted lines is the valve ring.

Z. *Typical Septal Flap of Tricuspid Valve Section.* N, right auricular myocardial wedge; O, septum fibrosum; P, bundle of His; Q, interventricular septum; R, right auricular endocardium; S, left auricular endocardium; T, valve spongiosa dipping into chorda tendinea insertion; U, valve fibrosa.

Area enclosed by dotted lines is the valve ring.

cardial and pericardial wedges which are, as it were, pressed in between them (Fig. 6). Other contributing irregularities in structure are the juxtaposition of the pulmonary myocardial conus and the presence of the *Septum Fibrosum* situated below the commissure between the right and posterior aortic cusps on the left side and the septal and anterior tricuspid leaflets on the right side.

As is well known, the aortic media is rich in elastic fibers. These exist as more or less parallel concentric sheets with, here and there, communicating transverse bars which include between them smooth muscle cells and connective tissue. As the media approaches the base of the aortic cusps it loses all but the most delicate of its elastic fibers and thus becomes the aortic *Annulus* (Fig. 1). Lewis and Grant have shown that for the greater part of the cusp, and in general, the aortic media ends in a wedge-shaped process whose apex is superficial to the *Annulus*. In the commissural region, however, the reverse of this takes place. They also found that in congenital bicuspid aortic valves the region of the fusion or absence of a commissure presents alterations in these relations. We have been able to confirm this and have noted in addition that as one approaches the commissures the wedge-shaped aortic termination changes into a more or less square pattern (Fig. 2) which again transforms itself into a wedge, now situated on the adventitial side of the aorta (Fig. 3). Furthermore, the commissural region is often marked by a slight thickening of the aorta at the level of the free edge of the cusps. Other parallel changes to be found in this region will be discussed in connection with the description of the *Sinus Pocket** to be given. Below the commissural junction the *Annulus* acquires elastic fibers and smooth muscle on its superficial (intimal) surface and thus again assumes the structure of aorta.

In the case of the *Right Aortic Cusp* (except that portion situated above the *Septum Fibrosum*) the aortic media generally changes into *Annulus* a short distance above the latter's insertion into the interventricular septum (Fig. 1). This insertion takes place by means of finger-like connective tissue processes which interdigitate with myocardial bundles. The main portion of the *Annulus*, however, makes a more or less obtuse-angled bend transversely and downward,† and

* The more or less transverse portion of *Annulus* and intimal covering which lines the bottom of the sinus we designate *Sinus Pocket* (Fig. 5).

† In these descriptions, "transversely" will be considered in reference to the main anatomical axis of the heart; "downward" is synonymous with "toward the apex."

riding atop the interventricular septum makes another bend abruptly cephalad as it reaches the ventricular cavity. The region of the cephalad bend is the *Ring*. Here fibers of the *Annulus* split off into two bundles to surround the *Ring Spongiosa*. The bundle which rounds the *Sinus Pocket* and enters the valve flap to make up its main support, the *Fibrosa*, is generally the larger one. The smaller bundle courses under the *Ring Spongiosa* and continues for a short distance down the inner surface of the ventricle under the endocardium, as a thin fibrous tissue sheet which we call the *Subvalvular Annulus*. The endocardial layer clothing the *Subvalvular Annulus* generally shows smooth muscle cells on section. It is to be observed that for the greater part of its insertion the *Ring* of the right aortic cusp sits on myocardium and has no direct connection with the pericardial wedge. However, here as in all other semilunar cusps, the *Ring* insertion lies almost directly over the pericardial wedge in the region of the commissure.

The line at which the *Annulus* meets the interventricular septum is generally clothed by pericardial tissue, usually fat, which serves to separate this arterio-annulus junction from the adjacent myocardium of the pulmonary conus, the latter being a direct muscular continuation of the interventricular septum. Particular attention should be paid to the presence of the pulmonary conus in this section, since it forms an absolute means of identifying this cusp. Furthermore, if the section is cut high enough, it is possible to distinguish the arterior half of the right aortic cusp from the posterior half. The former section contains the root of the pulmonary artery, the latter will show a cross-section of the right coronary artery.

It has already been mentioned that as one passes from the middle of the valve toward a commissure, alterations occur in the aortic media-annulus relations. In addition, it is to be noted that the *Sinus Pocket* formed by the *Annulus* becomes progressively smaller and the myocardium-annulus insertion line on cross-section assumes a horizontal direction instead of sloping from above downward (considered from the pericardial to the inner surface) as is the case in the middle of the cusp (Figs. 1, 2 and 3). These rules apply to all the aortic and pulmonary cusps.

With the following modifications, the description given for the topographic relations of the *Right Aortic Cusp* holds for the *Left Aortic Cusp* except the portion situated above the anterior mitral leaflet.

(*a*) In the case of the left aortic cusp (Fig. 4) the *Annulus* is inserted into left ventricular wall instead of interventricular septum.

(*b*) No pulmonary conus exists adjacent to the pericardial mantle.

(*c*) Smooth muscle is less frequently seen in the *Subvalvular Annulus* on section.

(*d*) If a block is cut through the middle of the valve, the left coronary artery or one of its branches will be seen embedded in pericardium not far from the arterio-annulus junction. If a section is cut more anteriorly, *i. e.*, close to the left-right commissure, the base of the pulmonary artery will be included; if cut more posteriorly, *i. e.*, close to the left-posterior commissure, the topographic relations will be similar to those to be described for the posterior cusp.

The topographic relations of the remaining portion of the left cusp, of the *Posterior Cusp* (Figs. 5 and 6), and of that part of the right cusp situated above the *Septum Fibrosum* are different in one important respect. Here, the aortic *Annulus* makes no bend as described above, but instead continues down to form the *Septum Fibrosum* or the *Fibrosa* layer of the anterior mitral leaflet, as the case may be. Because of this difference the corresponding aortic cusp *Rings* are brought much closer to the pericardial wedge, often being seated directly thereon instead of on myocardium. The pericardial wedge extends for a variable distance between *Annulus* and auricular myocardium in the case of the last-mentioned aortic cusps, at times passing below the level of the corresponding aortic *Ring*.* From this description it will at once be grasped that there is a strategic proximity between these aortic *Rings* and the pericardium, so that an infection originating in the former could easily pass by contiguity into the pericardium, and *vice versa*.

In addition to the differences noted above we may mention that in the case of the posterior aortic cusp, the arterio-annulus junction is usually low, at times forming part of the *Sinus Pocket*.

Summarizing briefly these topographic relations of the aortic cusps, it is seen that they divide themselves into four types by which it is possible to recognize histologically the cusps from which the sections are cut as follows.

(*a*) The main portion of the right cusp has the "U" type of insertion in which the aorta forms one arm of the "U," the cusp the other arm, and the base of the "U" sits on the interventricular septum and forms the bottom of the *Sinus Pocket*. In this "U" type of insertion the valve *Ring* sits on myocardium

* Occasionally the Arteria Anastomotica Magna (Kugel) may be seen in the pericardial wedge.

through the intermediary of a band of *Annulus*. The arterio-annulus junction is clothed by pericardium and the pulmonary myocardial conus lies adjacent to it.

(*b*) The main portion of the left cusp also has the "U" type of insertion. Here, the distinctive features are the absence of adjacent pulmonary conus and the frequent presence of coronary artery embedded in free visceral pericardium.*

(*c*) Continuing the scheme of using letters to represent physical configuration it may be said that the main portion of the posterior aortic cusp has the "Y" type of insertion. Here, the aortic *Annulus* continues as anterior mitral valve *Fibrosa* and forms the stem of the "Y." The posterior aortic *Ring* usually sits on pericardial wedge.

(*d*) The portions of right and posterior cusps participating in the right-posterior commissure also have the "Y" type of insertion but here the *Annulus*, in the form of the *Septum Fibrosum* (stem of the "Y"), eventually inserts into interventricular septum. The insertion is by means of connective tissue bundles interdigitating with myocardium.

Histology

For practical purposes the description which follows corresponds to an average aortic cusp of between the third and fourth decades. The age period changes will be briefly commented on below.

Ventricularis: The aortic cusp is clothed on its ventricular aspect by a fibro-elastic layer of varying degrees of density (Figs. 1 and 9). This layer, which we propose to call *Ventricularis*, is virtually a continuation of the ventricular endocardium, except for the posterior and left aortic cusps, where it is a continuation of the *Ventricularis* of the anterior flap of the mitral valve. This will be taken up subsequently. Histologically, the *Ventricularis* is seen to be covered by a single row of flat endothelial cells with round or oval nuclei and to consist of vertically (*i.e.*, parallel with the long axis of the body) directed connective tissue fibers interspersed with delicate elastic lamellae. The latter tend to become thicker and bunched together toward the ventricular surface, thus forming the *Ventricularis Elastica*.

It has been claimed by various authors that the elastic fibers in the valves do not appear until after birth. Nevertheless, we have found them in the *Ventricularis* at the proximal portion of the valve flap in 19 cm. (crown-rump) fetuses.

* The term "free visceral pericardium" denotes the absence of adjacent cardiac structure. Note that the left aortic cusp is the only one which shows free visceral pericardium on section at the *Ring* level.

The ridge constituting the line of closure of the aortic cusps consists of an abrupt thickening of the *Ventricularis* layer. This thickening is made up of closely packed vertically directed fibers of connective tissue interspersed with elastic fibrillae. The whole structure rests on a somewhat condensed band of *Elastica* which separates it from the remainder of the valve substance. In the center of the valve this closure line assumes nodular proportions and is called Nodulus Arantii (Fig. 7). In advancing age periods these Noduli become increasingly prominent, hardened and elastified threads often breaking off them at one end to float as streamers in the blood current. These form the so-called Lamblian excrescences. They are of interest in that they have been confused with healed verrucae.

The *Ventricularis* as a whole is rather poor in cells, though fibroblasts and occasional large mononuclear cells with scanty protoplasm can be seen scattered throughout it. These cells are more plentiful in the earlier age periods. This decreasing cellularity with increasing age is found in all the valve layers of the semilunar, as well as auriculoventricular, cusps.

Spongiosa: It has already been mentioned that the backbone of each valve leaflet consists of a dense collagenous layer and that in the aortic cusps the ventricular aspect of this layer gives way to a much looser structure which we designated *Spongiosa*. In a fetus of crown-rump length 12 cm. the *Spongiosa* already begins to be discernible. In the average adult of the second and third decade it consists of a very loose connective tissue layer which extends from the *Ring* (*Ring Spongiosa*) for a variable distance almost to the tip of the valve (Fig. 6). At the *Ring* the *Spongiosa* has a triangular shape limited below by *Annulus*, above by *Fibrosa* and toward the ventricular surface by *Ventricularis*. Interspersed in the loose connective tissue of the *Spongiosa* with its scarce fibroblasts and other mononuclear cells there can be found irregular clumps of dense collagen fibers, as well as a delicate feltwork of elastic fibrillae which are continuous on the one hand with those springing from the *Ventricularis* and on the other hand with rare elastic fibrillae running between the collagenous bundles of the *Fibrosa* (Fig. 9).

Fibrosa: Under the heading of "Topography" it has already been mentioned that the root of the aorta loses its musculature and most of its elastic fibers, thus transforming itself into *Annulus*, and that much of this collagenous *Annulus* curves around the *Sinus Pocket*

(Valsalva) to ascend the valve leaflet as its *Fibrosa*. In rounding the *Sinus Pocket* the collagenous bundles change their general direction and structure. In the *Annulus* they are in the form of interlacing whorls. Under the *Sinus Pocket* the superficial bundles take on a course more or less parallel to the bottom of the "U" and, as the *Fibrosa* proper, they run mainly in a transverse direction.

In the crypts between the collagenous bundles there are to be found very delicate elastic fibrillae, as well as mononuclear cells with round dense nuclei and rather scant protoplasm. The *Fibrosa* ascends toward the tip of the cusp. In most instances, however, the arterial aspect of the tip of the *Fibrosa* consists of a much looser, at times myxomatous, structure, not unlike the *Spongiosa* in character (Fig. 1).

The *Fibrosa* is more or less sharply limited on its ventricular aspect by the looser *Spongiosa* and on its arterial aspect by the delicate *Arterialis* about to be described.

Arterialis: As one follows the more superficial elastic fibers down the intima of the aorta toward the cusps, they are seen to fuse into one or more bands which round the *Sinus Pocket* superficially and ascend the arterial surface of the aortic cusps as a delicate elastic layer. This rapidly thins itself out, often disappearing about one third of the way up the valve. Before progressive (hyperplastic) changes have taken place, this elastic lamella is clothed by a layer of flat endothelial cells and is separated from the adjacent *Fibrosa* by a very sparse, loose connective tissue containing extremely delicate elastic fibrillae, as well as mononuclear cells poor in cytoplasm with darkly staining nuclei. Normally, and in younger individuals, the endothelial cells sit practically directly on this elastic layer.

As has been mentioned before, the *Arterialis Elastica* is shorter and far more delicate than the corresponding *Ventricularis Elastica* (Figs. 5 and 9).

Pulmonary Valve

Topography

The topography of the pulmonary cusps is very simple. The general plan is the "U" type of insertion, as described for the aortic cusps. Because of this there is no direct insertion of pulmonary *Ring* on pericardium, except at the commissural junctions. As in the

aorta, the metamorphosis of pulmonary artery into *Annulus* takes place at, or slightly above, the level of the subjacent ventricular myocardium. The relations of pulmonary artery to *Annulus* are similar to those described for the aorta and the rules laid down concerning the slope of the *Annulus* and the changes which take place on approaching the commissure hold for the pulmonary cusps.

TABLE I

Comparison of Histological and Topographical Features of Pulmonary and Aortic Cusps

Pulmonary Cusps	*Aortic Cusps*
* Pulmonary artery delicate.	* Aorta thick.
* Various layers delicate.	* Layers on the whole thick.
Fibrosa frequently shows transverse ridge-like projections into *Sinus Pocket*.	Ridge-like projections less frequent.
Ring Spongiosa often small and fibrillar. Not infrequently absent from section.	*Ring Spongiosa* generally larger. Seldom fibrillar, more often spongy. Seldom absent from section.
Valve Spongiosa less frequently definite. Character similar to *Ring Spongiosa*.	*Valve Spongiosa* sharply defined when present. Character similar to *Ring Spongiosa*.
Smooth muscle in *Ventricularis* rare in any cusp section.	Smooth muscle in *Ventricularis* not infrequent, especially in *Ventricularis* of right aortic cusp.
* *Subvalvular Annulus* is absent or very delicate.	* *Subvalvular Annulus* conspicuous.
* Interdigitations of *Annulus* with myocardium generally delicate.	* Interdigitations coarser, increasing with age periods.

(The items marked by asterisks demonstrate the more characteristic differences.)

The main differences between the pulmonary and aortic valve sections are brought out in Table I. The items marked by asterisks demonstrate the more characteristic differences. In addition to the points brought out in the table, it is to be noted that in contrast to the aortic valve where the left cusp section is the only one to show "free visceral pericardium," the latter is present on the anterior and right pulmonary cusps.

The individual characteristics of the several pulmonary cusps are as follows.

The *Left Cusp* (Fig. 11) is inserted on to the anterior portion of the interventricular septum; as a consequence of this a section through the middle of this cusp shows the double myocardial crest characteristic of the latter. If cut more toward the left, it will show a large transverse section of ventricular wall, possible with visceral pericardium. If cut toward the right, adjacent aortic root will be seen. These sections show no subvalvular *Annulus* but are apt to have left coronary artery present.

The *Anterior Cusp* (Fig. 12) is seated on the delicate anterior wall of the pulmonary conus. In this region the pericardial mantle is generally very thin. If subvalvular *Annulus* is present in the pulmonary valve it will generally be found in the anterior cusp section as a delicate layer extending several millimeters below the valve *Ring*. This, together with the very slender visceral pericardial layer mentioned above, is characteristic of the cusp.

The *Right Cusp* (Fig. 13) is seated on the more supple right wall of the pulmonary conus. Here the pericardial fat is generally quite thick. These features and the absence usually of *Subvalvular Annulus* are characteristic of this cusp. If a section is cut more posteriorly, adjacent aorta may be seen.

Histology

The histological differences between pulmonary and aortic cusps are much less obvious than are the topographic. In general, as mentioned before, the layers are more delicate in the former. The *Ventricularis* and *Arterialis* layers are generally so thin as to make the *Fibrosa* more conspicuous by contrast.

Whereas in the aortic cusps the *Ring* and *Valve Spongiosa* are, as it were, sharply scooped out of the *Fibrosa* and filled with a loose jelly-like tissue made up of transversely coursing collagen and elastic fibrillae with stellate cells, in the pulmonary cusps the separation of this layer from the *Fibrosa* is usually not so sharp, fragments of collagen being scattered throughout (*cf.* Figs. 6 and 12). Elastic fibrillae are not seen so often until later age periods and the connective tissue feltwork is often denser.

In later age periods the prominent Noduli Arantii of the aortic valve form a characteristic difference from the corresponding, more delicate, so-called Noduli Morgagni of the pulmonary cusps.

General Considerations Concerning the Auriculo-ventricular Valves

It is a relatively simple matter to set definite limits to the extent of the semilunar valve cusps. In a previous paragraph it was suggested that the valve *Rings* should be considered to constitute their proximal end. This gives one a fairly concrete point of departure, inasmuch as the valve *Ring* itself is almost invariably limited proximally by subjacent *Annulus* (Fig. 1). In any event, shifting the imaginary line of semilunar cusp insertion slightly one way or another is, in so far as one can see, of no great moment.

This, however, is distinctly different in the case of the auriculoventricular cusps for, as indicated earlier in this paper, one's conception of the limits of the cusps inevitably carries with it the question of the existence of myocardium, and because of this, of blood vessels in the valve. A glance at a typical cusp (Fig. 6) will make this point clear. If arrow "A" were to be considered the proximal end of the cusp, the auricular myocardial wedge would be included and it could therefore be said on this basis that many of the auriculoventricular cusps have myocardium. Further, since myocardium is always supplied by blood vessels, it follows according to this definition that these auriculoventricular cusps possess blood vessels. If, on the other hand, arrow "B" is to be considered the proximal end of the cusps (and this is the definition which we favor), the myocardium and its blood supply ceases to be a factor in the argument and the question of the existence of blood vessels in human heart valves involves consideration of only the fibro-elastic portion — an entirely different matter as will be seen. It seems to us that this is the crux of the discussion on whether myocardium exists in the auriculoventricular valves and it is on this very point that a large and confusing literature has sprung up. Various authors have chosen arbitrary limits for the origin of the auriculoventricular valves, particularly of the anterior mitral cusp, and have reported abundant or sparse auricular musculature extending for a greater or lesser distance down the valve according to their conception as to whether

the base of the valve was higher or lower in the auricle (Kürschner, Reid, Joseph, Gussenbauer, Beitzke, Hoessli, Tandler, De Castro). The significance of the relatively infrequent occurrence of ventricular musculature in the base of the tricuspid valve will be discussed later.

It is obvious from what has been said that the presence or absence of myocardium in the auriculoventricular valves must be settled before one can intelligently discuss the existence of blood vessels in valves. Furthermore, inasmuch as the latter question is intimately bound up with the embolic theory of endocarditis, a clear definition of terms becomes not only desirable but absolutely essential. When one considers the question of the pathogenesis of valvulitis one is primarily concerned with the membranous structure. This very practical reason alone should justify a definite limitation of the valve to the fibro-elastic portion. But there are other reasons as well. We have seen that the valve *Ring* is a natural and simple limit to be set for the extent of the semilunar cusps. It is desirable that, if possible, a uniform conception be held for all valve cusps and that therefore the extent of the auriculoventricular valves should be limited in the same manner. Furthermore, it does not seem to be more unreasonable to exclude auricular or ventricular myocardium from our definition of valve leaflet than it does, for example, to exclude sclera from our conception of what constitutes cornea. Apparently, Weber and DeGuy appreciated this source of confusion in the case of the anterior mitral cusp and attempted to circumvent the difficulties by naming the entire structure "la région mitro-aortique" and dividing it into three zones, *viz.*: (1) la zone supérieure ou sigmoidienne . . . limitée . . . en bas par trois lignes . . . d'insertion des valvules aortiques; (2) la zone moyenne ou mitro-auriculaire, limitée . . . en bas *par une ligne traversale correspondant à la partie terminale des fibres musculaires venant de l'oreillette* . . . ; and (3) la zone inférieure ou grande valve mitrale. . . .

In view of all the considerations mentioned above and for many equally important reasons which need not be entered into here, we propose to use the valve *Ring* to set the proximal limit of the auriculoventricular cusps. Since all the auriculoventricular cusps have inserted into them a wedge of auricular myocardium, a line drawn through the apex of this wedge at right angles to the auricular endocardium may be considered an excellent and simple means of defining in turn the proximal limit of the auriculoventricular valve

Rings (Text-Fig. 2). In the case of all the auriculoventricular cusps, excepting the septal flap of the tricuspid, the *Ring* may be arbitrarily considered as an inverted pyramid-shaped portion of *Annulus* in the immediate vicinity of the auricular myocardial wedge apex, limited above by the line referred to, which constitutes the base of this inverted pyramid, and internally by the endocardium. We have found it convenient to consider the pyramid more or less equal-sided and have taken the width of the valve as a rough measure of each side. In contrast to all the other auriculoventricular valve cusps, the septal flap of the tricuspid, as will be noted in Fig. 16, is generally inserted onto *Annulus Fibrosis* or interventricular septum through the intermediary of a wide base. Here the *Ring* may be conveniently considered as roughly rectangular in shape. The upper limit is the transverse line drawn through the apex of the auricular wedge; the lower limit is a similar line drawn at right angles to the endocardium at the level of the *Sinus Pocket*; the outer limit is in an imaginary line drawn parallel to the endocardium at a distance from it approximately equal to the width of the valve; the inner limit is the endocardium.

Contrary to what is found in the semilunar valves, the auriculoventricular cusps seldom have a clearly delimited valve or *Ring Spongiosa*. In the region of the apex of the auricular myocardial wedge the *Fibrosa* generally shows a looser structure of its collagenous bundles. For the sake of uniform terminology this layer should also be referred to as *Spongiosa*. It may at times be seen extending along the auricular aspect of the *Fibrosa* toward the valve tip (Figs. 8 and 16). The layer is generally more conspicuous opposite the insertions of the chordae tendineae where it can be seen at times dipping into the latter in the form of a wedge. Both here and at the *Ring* the *Spongiosa* may acquire considerable fat during the course of the degenerative involutionary changes attendant on later age periods.

The most conspicuous feature of the auriculoventricular valve structure is the extremely dense, broad *Fibrosa* which constitutes the bulk of the cusp. As in the semilunar cusps, the *Fibrosa* not infrequently transforms itself into a looser, sometimes myxomatous, structure as it approaches the tip. The chordae tendineae may be considered as dense collagenous prolongations of the *Fibrosa* layer which link the latter to the papillary muscles or ventricular myocardium.

In leaving the *Fibrosa*, the chordae tendineae carry with them a light mantle of the *Ventricularis* covering the valve leaflet.

With the above mentioned points in mind, the typical auriculoventricular cusp section shows the following topographic features (Fig. 9).

An auricular portion consisting of endocardium and subjacent myocardium ends in a wedge.

Linking this wedge to ventricular myocardium and interdigitating with the latter there is a dense collagenous *Annulus*.

On the external surface, pericardium clothes this auriculoventricular junction and sends a wedge of adipose tissue to insert into the *Annulus*. The proximity of this pericardial wedge to the valve *Ring* and the frequency with which the latter is the seat of inflammation are matters of considerable interest from the point of view of pathogenesis.

The *Annulus* merges imperceptibly with the valve *Ring* at the level of the myocardial wedge.* The *Ring* in turn gives way to valve *Fibrosa* and this continues for a variable distance toward the valve tip.

The auricular endocardium continues beyond the myocardial wedge tip down the valve as its *Auricularis* layer. Similarly, too, the ventricular endocardium passes under the *Valve Pocket* clothing the ventricular surface of the *Annulus*, rounds the latter and spreads itself over the outer surface of the cusp as the *Ventricularis*.

Mitral Valve

Topography

The topography of the typical auriculoventricular valve has already been described and the description given is directly applicable to the *Posterior Mitral Cusp* (Fig. 8). A section through this region shows the characteristic dome-shaped crest of the left ventricular wall, the thick left auricular endocardial layer, the not infrequent passage of the left auricular myocardial wedge below the level of the ventricular crest and the presence, generally, of left coronary circumflex artery in the *Free Pericardium* of the auriculoventricular groove. At times a short subvalvular extension of the *Annulus* may be seen on the ventricular wall below the valve pocket.

The *Anterior (Aortic) Mitral Cusp* is unique in structure (Fig. 6). In describing the posterior aortic cusp it was mentioned that it falls into the category of the "Y" type of *Annulus* insertion, *i. e.*, the

* At times the auricular myocardial wedge may send several finger-like strands of tissue for as much as 1 cm. down the valve cusp. This places the *Ring* unusually low, but it is better so considered than to change the definition and thus confuse more important issues.

aortic *Annulus* continues down the ventricular face of the auricular myocardial wedge and after reaching the tip of the latter becomes successively anterior mitral cusp *Ring* and valve *Fibrosa*. The portion of *Annulus* which extends between the aortic and mitral *Rings* we call *Mitral-Aortic Intervalvular Fibrosa*. It is well to note that this structure belongs neither to aortic nor to mitral cusps. For embryogenetic and anatomical reasons this, like the myocardial wedge, should be considered part of the auricle.

Further points of importance are the characteristic auricular myocardial wedge clothed by thick left auricular endocardium and the typical structure of aortic cusp (left or posterior) superimposed.

Histology

Auricularis: As is well known, the left auricular endocardium is made up of dense fibrous and elastic sheets interspersed with smooth muscle bundles which are generally situated close to the subendocardium. As the elastic fibers are traced toward the mitral valve it is seen that the outermost lamellae (*i. e.*, those toward the subendocardium) tend to fuse into a more or less condensed stratum. In the region of the apex of the auricular myocardial wedge many of the remaining elastic lamellae fuse into this lower condensed stratum. In the later age periods, the innermost elastic sheets also show condensations which fuse with the subendocardial strata near the auricular myocardial wedge apex. The net result is that the number of elastic lamellae is considerably reduced and concentrated. This concentration zone continues down the auricular surface of the mitral valve for a variable distance toward the tip. Interspersed among the elastic fibers there can be seen stretches of collagen.

In the case of the anterior mitral cusp, a continuation of the endocardial smooth muscle can usually be traced down the *Auricularis* layer, clothed by *Auricularis* elastic lamellae. The smooth muscle can become so prominent as to form, in some cases, approximately one-fifth of the entire thickness of the valve leaflet. For about two-thirds of the way down the cusp the elastic lamellae can be seen to lie almost immediately beneath the endothelial-covered surface. However, as the closure line of the valve is approached at times the bulk of the elastic lamellae begins to occupy a slightly less superficial position, being covered by a thicker and wider band of

dense connective tissue which may in turn carry more delicate strands of elastic tissue.

The most superficial covering of the auricular surface of the valve is the endothelial layer referred to above. This consists of flat cells with scanty protoplasm and moderately dense nuclei. Between this endothelial layer and the most superficial elastic band there is an almost imperceptible jelly-like zone containing an occasional mononuclear cell.

The tips of the mitral leaflets are generally of a more gelatinous, embryonal consistency (Figs. 6 and 8). The *Auricularis* layer described, together with the others about to be described, merge imperceptibly with this loose embryonal tissue of the valve tip. The latter consists of a network of stellate cells with scanty protoplasm lying in a matrix poor in collagenous fibrillae and staining at times faintly bluish with hematoxylin. As in the semilunar cusps the *Auricularis* layer is relatively poor in cells.

Spongiosa: The left auricular subendocardium consists of dense bundles of collagen interspersed with thick elastic fibers. A continuation of this structure mingles with the outer looser layer of the *Fibrosa* to form the essential substance of the mitral valve *Spongiosa*. In the case of the posterior cusp the structure is generally much looser than that of the anterior and, as mentioned before, opposite the insertions of chordae tendineae it may assume an appearance which is almost indistinguishable from that seen in the semilunar cusp *Spongiosa*. That is, small collagenous bundles may be seen irregularly interspersed with a feltwork of delicate elastic fibrillae, a very loose ground substance and scatterings of mononuclear cells. In the region of the *Ring* the elastic fibrillae are generally coarse. At or near the cusp tip the *Spongiosa* layer merges imperceptibly with the other layers.

Fibrosa: In the case of the *Posterior Mitral Cusp*, it has already been mentioned that the *Annulus* interdigitates with myocardium and generally continues as an extension under the ventricular subendocardium. These collagenous fibers, on the whole, run parallel to the ventricular surface, round the valve pocket and continue into the valve substance to form the *Fibrosa* layer (Fig. 8). Here the fibers run parallel to the chordae tendineae. Indeed, they form an unbroken continuation with the main body of the latter. The collagenous *Fibrosa* which is dense and shows mononuclear cells with scant pro-

toplasm lying between its bundles is generally almost entirely free of elastic fibrillae (Fig. 10). It extends toward the cusp tip where it becomes continuous with the spongy structure referred to above.

The *Fibrosa* of the *anterior mitral cusp* is a direct continuation of the *Mitral-Aortic Intervalvular Fibrosa* and the general direction of its fibers is transverse.

Ventricularis: In the case of the *Posterior Mitral Cusp*, the endocardial fibro-elastic covering of the ventricle curves round the valve pocket and descends for a variable distance toward the cusp tip forming its *Ventricularis*. The elastic fibers are generally considerably more delicate and shorter than the corresponding ones on the *Auricularis* layer. They are covered by the same type of endothelium as described for the *Auricularis* layer and between the elastic lamellae and the *Fibrosa* there is to be found a small amount of relatively acellular loose gelatinous tissue. Under certain conditions the *Ventricularis* layer may become somewhat thickened.

In the case of the *Anterior Mitral Cusp* (Fig. 6), this layer is a direct continuation of the subaortic endocardial tissue, the latter in turn being a prolongation of the semilunar cusp *Ventricularis*. Furthermore, in this cusp the *Ventricularis* shows many dense bundles of elastic tissue, at times much more conspicuous than the *Auricularis* bundles. This is particularly well seen in the younger age periods where the *Ventricularis Elastica* carries with it a very definite structure of longitudinal running collagenous bundles. As in the case of the mitral posterior, this *Ventricularis Elastica* is generally shorter than the *Auricularis Elastica*.

Tricuspid Valve

Topography

The topography of the tricuspid leaflets follows closely the general description given for the auriculoventricular valves. However, they can be very easily distinguished from the mitral flaps because of their greater delicacy in structure and other peculiarities of their own about to be described. As in the case of the mitral cusps the general plan is that of an auricular endocardial and myocardial wedge which is joined to a ventricular crest through the intermediary of *Annulus*, the tricuspid cusps springing from this *Annulus*.

The *Anterior Flap*, the longest of the three, shows an extremely

delicate auricular endocardium with scattered smooth muscle cells. The peculiarity of the ventricular crest easily distinguished this section because the myocardium tends to assume a trough shape, the concavity of which points toward the auricles (Fig. 14). A rather abundant free pericardial wedge fills in the space between this trough and the right auricular myocardial wedge. This wedge carries the right circumflex coronary artery. A few strands of subvalvular *Annulus* can occasionally be seen in the section.

The *Posterior Tricuspid* section differs from the anterior in several respects. Here, the auricular endocardium retains the slender structure characteristic of right auricle but is generally thicker than that seen in the anterior tricuspid section. The smooth muscle cells are generally more abundant. While the septal crest is seldom as rounded, dome-shaped and regular as in the mitral posterior section, the characteristic trough structure of the anterior tricuspid is rarely present — a more bulky, often irregular dome-shaped myocardial crest taking its place (Fig. 15). The separation between the auricular and ventricular myocardium is generally more marked in this section, the *Annulus* showing a neck-like extension. At times this neck-like extension may consist partly of myocardial bundles. There is seldom *Subvalvular Annulus* to be seen. The pericardial wedge is moderate in amount and generally carries a branch of coronary artery. The flap itself is shorter than the anterior.

The *Septal Flap* is the shortest of the three and its situation on the septum is so characteristic that one can easily distinguish this cusp from the others, as well as determine the particular point at which the section was cut (Fig. 16). The most anterior portion of this cusp is inserted on *Septum Fibrosum* so that the *Annulus* of this cusp becomes continuous with it. Since, further, the *Septum Fibrosum* is also continuous with the *Annulus* of the aortic cusps, there exists thus a collagenous linkage between mitral, aortic and tricuspid *Rings*. As one traces the septal cusp backward, however, its insertion line passes onto the top of the interventricular crest and occupies a progressively lower (caudad) site as it extends posteriorly. Thus, by estimating the approximate distance between the tricuspid *Ring* and the top of the septal crest, one can fairly well establish the position of the section.

The auricular endocardium is generally of about the same structure as that seen in the posterior tricuspid section. The auricular

myocardium ends in a wedge whose apex is superficial to the *Septum Fibrosum*. The general slope of the latter is usually from above downward, considered from left to right.

In the region of the *Septum Fibrosum* the ventricular myocardium ends in a fairly regular dome-shaped crest. As one proceeds backward, however, the dome shape can at times be seen to be replaced by a sloping surface whose highest point is toward the left ventricle. It will be seen from this that the general slope of the *Septum Fibrosum* is also continued in the myocardial portion of the interauricular septum.

The pericardial wedge as such is present in an abortive form only in the posterior sections of this cusp where fragments of fatty tissue can be seen to surround the auriculoventricular node. The continuation of the latter in the form of the bundle of His can be readily seen in this section. As in the other two tricuspid sections, *Subvalvular Annulus* is inconspicuous. In the case of all three sections the ventricular myocardial interdigitations are slender.

Histology

The histology of the tricuspid leaflets is similar to that of the mitral valve posterior. The following features, however, should be noted.

The endocardium of the right auricle shows a considerable abbreviation of structure over that of the left auricle. It is generally thinnest over the anterior cusp. The latter tends to be rather dense in structure and shows the looser myxomatous tip less frequently than the other cusps. The general direction of the collagenous bundles is similar to that described for the mitral posterior cusp. There is often a greater difficulty in discerning the *Spongiosa* layer in the tricuspid cusps. The general distribution of the elastic lamellae and the cytology shows no fundamental differences from what has already been described. One peculiarity of the tricuspid leaflets to which attention has already been drawn in the literature is the fact that ventricular myocardial bundles are not infrequently found inserted fairly low into the base of the valve. This is particularly true for the posterior cusp and is found much less frequently in the septal cusp. In spite of the numerous reports published on this point it does not seem that the findings of these muscular bundles is of ap-

preciable significance. The bundles generally do not descend deep into the cusps and are nothing more than a slight exaggeration of the natural infolding of the ventricular myocardial tube with that portion of the auricular canal which is concerned with the formation of auriculoventricular cusps. The same explanation accounts for the not infrequently low position of the auricular myocardial wedge in these sections.

The Question of the Existence of Blood Vessels in the Valves

It will be noted that in the description of the various valves given in this report blood vessels were not mentioned. We have deliberately avoided taking up the very controversial question as to the existence of blood vessels in normal valves largely because it can not be done justice, without going into very lengthy detail. Briefly considered, it may be said that there are three schools of thought on this subject: One group holds that human heart valves have no blood vessels; another believes that human heart valves are supplied with blood vessels in at least a considerable proportion of cases and that failure to demonstrate them by many observers is due to faulty technique; the third group believes that blood vessels occur at times in postnatal human heart valves and that when they occur they probably represent fetal remnants.

Without desiring to enter into controversy here, we may say that we are rather doubtful at present as to whether blood vessels occur, at any rate with any degree of frequency, in *normal* human heart valves, providing we have a very clear understanding as to what we consider *normal*.

In previous reports evidence was adduced by us which pointed to the fact that in at least 2 per cent of a group of 700 individuals who came to autopsy in the general hospitals from which our material was obtained, blood vessels were found in some valves of what appeared to be otherwise normal hearts. Our present scepticism is due to the fact that the results of our more recent investigations have caused us to doubt the normality of many of the hearts which we previously considered normal. Furthermore, as indicated in the description given above, our definition of the *Ring*, which is the proximal termination of the valve cusp, automatically excluded myo-

cardium. Considered in this way, and we suggest that this is a logical and simple definition, the question of the existence of blood vessels in valves ceases to be one of major importance. Certainly if they occur at all they are most infrequent.

It may be said in passing that the practice of comparing human heart valves to those of lower animals is one which is very prone to lead one into erroneous conclusions.

The Question of the Existence of Blood Vessels in the Rings

In describing the various valve *Rings* we attempted to picture the cellular and tissue components which make up their structure. No mention was made of the presence or absence of blood vessels in these sites. This omission was deliberate and was done in order to simplify the description. Actually, in a statistical analysis made for this purpose on 100 hearts from various age periods, capillaries only were found in the *Rings* with the following frequency:

Anterior mitral valve *Ring*	1 per cent
Posterior " " "	2 " "
Aortic " "	0 " "
Tricuspid " "	14 " "
Pulmonary " "	7 " "

Excluding the tricuspid *Ring* from consideration for a moment, it may be stated that in not a single heart were vessels found in more than one of the other *Rings*. This fact, together with others to be discussed, seems to us to be of considerable importance.

It will be observed that capillaries are not infrequently found in the tricuspid *Ring* (14 per cent), particularly in the septal flap *Ring*. These capillaries are generally in the form of rather large thin-walled channels. They do not resemble granulation tissue capillaries in any respect.

The capillaries found in the other *Rings* are generally few, usually near to the subjacent myocardium, small and circular on cross-section, not surrounded by inflammatory cells, and in structure easily differentiated from granulation tissue capillaries.

It will be obvious from the above description that the great infrequency of capillaries, particularly in the mitral and aortic *Rings*, their relative infrequency in the other *Rings*, their normal appear-

ance, the absence of inflammatory cells, the fact that the occurrence of capillaries in more than one *Ring* at the same time (excluding the tricuspid) must be very rare, together with the absence of arterioles or large vessels in these sites, make any definite deviation in these findings strongly suggestive of the occurrence of a pathological lesion past or present.

Holsti examined twenty-two hearts from cases of sudden death and found capillaries in the valve "roots" (Wurzel) of eight cases distributed as follows: eight in the mitral, six in the tricuspid and two in the aortic. The author, however, speaks of finding myocardium in the "roots" of many of his cases which obviously makes his definition of "root" different from our conception of what constitutes *Ring*. Furthermore, in eleven of the hearts, the author found definite evidence of valvulitis and in three cases marked vascularization of the "root" and valves as far as the tip. For these reasons it is impossible to compare statistically Holsti's findings with ours in respect to *Ring* vascularization.

While on this point it should be mentioned that a number of the hearts examined in our series showed inconspicuous scatterings of inflammatory cells. Often this was found in cases in which some form of sepsis had occurred during life. We did not mention these exudates in connection with our description of normal valves because they seemed obviously to be part of some pathological process, however slight.

Noduli Albini and Congenital Valve Hematomata

For completeness, mention may be made of the so-called Noduli Albini and of valve hematomata. According to Tandler, Cruveilhier was the first to describe the former. These consist of tiny jelly-like thickenings of the auriculoventricular valve substance and are to be found in the region of the insertions of the chordae tendinae of the first order. They are seen most frequently in the newborn and represent slight exaggerations of the myxomatous tip referred to in our previous sections.

The congenital valve hematomata consist of endothelial-lined spaces containing blood and are frequently found on the auricular aspect of the auriculoventricular cusps. These structures are seen only during the first year of postnatal life (Tandler). A number of

investigators believe them to be vestigial remnants of valve vasculature. Others consider them endocardial sack-like infoldings. Another possibility which we would like to suggest is that during the course of development endothelial cells may be invaginated into the substance of the cusp, become nipped off and, because of their angioblastic properties, develop aborted vessels or vascular cyst-like spaces. The contained blood cells themselves may be of similar origin.

Age-Period Changes in Valves

The human heart valves, like other structures in the body, continue to show evolutionary and involutionary changes after birth. What the significance of these changes may be in respect to the production of disease processes one can only surmise. It is well to bear in mind, however, that with advancing age periods the various layers of the valves previously described become progressively poorer in cells and take on the following changes: The strata become increasingly well defined; the semilunar cusp *Spongiosa* becomes more and more fibrous and elastified; the auriculoventricular valve *Ring Spongiosa* and the *Spongiosa* situated opposite the chordae tendineae insertions become loose and often the seat of fat deposits; the elastic membranes become heavier and longer; the *Auricularis* and often the *Ventricularis* layers become appreciably denser, more collagenous and thickened; and the collagenous *Fibrosa* undergoes degenerative lipoid changes. The point last mentioned is inevitably associated with the arteriosclerotic calcium salt deposition of the later age periods. Particularly is this true of the lime changes which occur in the *Annulus* of the valve *Rings* (Mönckeberg).

Summary

There has been described in this paper in some detail the normal histology and topography of the individual cusps of the valves in the human heart. It has been shown that these have a number of characteristics in common and yet sufficient individual differences to distinguish them from one another. The desirability of sharply delimiting the different cusps has been discussed and a method of accomplishing this has been suggested. This method is calculated to clear up the confusion which exists on the question of the presence of

myocardium in the auriculoventricular valves. A simple but comprehensive classification of the various valve layers has been given. In designing this classification there has been borne in mind the advisability of using a uniform nomenclature and of reducing the variations in valve structure to as few common denominators as possible. Attention has been drawn to the possible bearing which the various differences in valve structures may have on the development of pathological processes which occur at these sites. An indication has also been given of the evolutionary and involutionary postnatal changes which take place in the cusps.

The main purpose of the work here reported is to serve as a base line for further studies on disease of heart valves, because it is realized that an intimate knowledge of the structure of the valve cusps, together with their normal variations and age-period changes, is essential for the recognition and understanding of pathological processes.

BIBLIOGRAPHY

Albini. Ueber die Noduli an den Atrioventrikularklappen des Menschen. *Wochenbl. d. Zeit. d. k. k. Ges. d. Aerzte in Wien*, 1856, **2**, No. 26, 401, 405.

Albrecht, E. Der Herzmuskel und seine Bedeutung für Physiologie, Pathologie und Klinik des Herzens. Julius Springer, Berlin, 1903.

Aschoff, L., and Tawara, S. Die heutige Lehre von den pathologisch-anatomischen Grundlagen der Herzschwäche. Gustav Fischer, Jena, 1906.

Beitzke, H. Ueber die sogenannten "weissen Flecken" am grossen Mitralsegel. *Virchows Arch. f. path. Anat.*, 1901, **163**, 343.

Benninghoff, A. Handbuch der mikroskopischen Anatomie des Menschen, Möllendorff, W. Julius Springer, 1930, **6**, 171.

Bernays, A. C. Entwicklungsgeschichte der Atrioventrikularklappen. Morphologisches Jahrbuch, Gegenbaur, C., 1876, **2**, 478.

Coen, Edmondo. Ueber die Blutgefässe der Herzklappen. *Arch. f. mikr. Anat.*, 1886, **27**, 397.

Curtis, F. De la structure des valvules sigmoides de l'aorte et de l'artère pulmonaire. *Compt. rend. Soc. de biol.*, 1888, Ser. 8, 591.

Darier, J. Les vaisseaux des valvules du coeur chez l'homme à l'état normal et à l'état pathologique. *Arch. de physiol. norm. et path.*, 1888, Ser. 2, 35 and 164.

De Castro, Ugo. Disposizione anatomica e significato dei muscoli atrio-valvolari con speciale reguardo alla funzione delle valvole atrio-ventricolari del cuore (quotes early literature). *Cuore e circolaz.*, 1926, **10**, 441–467.

Dewitzky, W. Ueber den Bau und die Entstehung verschiedener Formen der chronischen Veränderungen in den Herzklappen. *Virchows Arch. f. path. Anat.*, 1910, **199**, 273.

Dreyfuss, M. Zur Histopathologie der Endokarditis. *Frankfurt Ztschr. f. Path.*, 1922, **27**, 527.

Fahr. Das elastische Gewebe im gesunden und kranken Herzen und seine Bedeutung für die Diastole. *Virchows Arch. f. path. Anat.*, 1906, **185**, 29–43.

Gross, L. The Blood Supply to the Heart in its Anatomical and Clinical Aspects. Paul B. Hoeber, New York, 1921.

Gross, L., Antopol, W., and Sacks, B. A standardized procedure suggested for microscopic studies on the heart. With observations on rheumatic hearts. *Arch. Path.*, 1930, **10**, 840–852.

Gussenbauer, C. Ueber die Muskulatur der Atrioventrikularklappen. *Sitzungsb. d. k. Akad. d. Wissensch.*, **57**, 186.

Henle, J. Handbuch der systematischen Anatomie des Menschen. Gefässlehre. Braunschweig, 1868, **3**.

Hoessli. Muskelreste im langen Mitralsegel. *Centralbl. f. allg. Pathol. u. path. Anat.*, 1910, **21**, 865–871.

Holsti, Olsen. Beiträge zur Kenntnis der entzündlichen Klappenaffektionen mit besonderer Berücksichtigung der Pathogenese. *Arb. a. d. path. Inst. zu Helsingfors*, Gustav Fischer, 1928, **5**, 401.

Joseph, L. Ueber die Ringe und Klappen des menschlichen Herzens. *Virchows Arch. f. path. Anat.*, 1858, **14**, 244.

Königer, H. Histologische Untersuchungen über Endokarditis. *Arb. a. d. path. Inst. zu Leipzig*, 1903, No. 2, 1.

Krause, W. Handbuch der menschlichen Anatomie. Hannover, 1876, **1**, 302.

Kugel, M. A. Anatomical studies on the coronary arteries and their branches. I. Arteria anastomotica auricularis magna. *Am. Heart J.*, 1928, **3**, 260.

Kugel, M. A., and Gross, L. Gross and microscopical anatomy of the blood vessels in the valves of the human heart. *Am. Heart J.*, 1926, **1**, 304.

Kürschner. (1) Ueber die venösen Klappen des Herzens und ihre Aktion. *Frorieps Neue Notizen*, 1840, No. 316.
(2) Herz und Herztätigkeit. Handwörterbuch der Physiologie mit Rücksicht auf physiologische Pathologie, R. Wagner, 1844, **5**.

Lambl. Papilläre Excrescenzen an der Semilunar-Klappe der Aorta. *Wien. med. Wchnschr.*, 1856, **1**, 244.

von Langer, L. (1) Ueber die Blutgefässe in den Herzklappen des Menschen. *Sitzungsb. der k. Akad. d. Wissensch.*, 1880, **208**, 81–82.
(2) Ueber die Blutgefässe in den Herzklappen bei Endocarditis valvularis. *Virchows Arch. f. path. Anat.*, 1887, **109**, 465.

Lewis, T., and Grant, R. T. Observations relating to subacute infective endocarditis. *Heart*, 1923, **10**, 21–29.

Luchsinger, B. Zur Architectur der Semilunarklappen. *Arch. f. d. ges. physiol. des Menschen u. d. Tiere*, 1884, **34**, 291.

Luschka, H. Die Structur der halbmondförmigen Klappen des Herzens. *Arch. f. Physiol. Heilk.*, 1856, 537.

Masson, P. Some histological methods. Trichrome stainings and their preliminary technique. *Internat. A. M. Museums Bull.*, 1929, **12**, 75–90.

Mönckeberg, J. G. Der normale histologische Bau und die Sklerose der Aortenklappen. *Virchows Arch. f. path. Anat.*, 1904, **176**, 472.

Nagayo, M. Zur normalen und pathologischen Histologie des Endocardium parietale. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1909, **45**, 283.

Odinzow, V. P. Die Vascularization der Herzklappen im Kindesalter. Inaug. Diss., München, 1904.

Poirier. Traité d'anatomie. Le coeur et les artéres. Paris, 1902, **2**, Ser. 2, 629.
Rappe, K. B. C. Ueber Gefässe in den Herzklappen. Inaug. Diss., Göttingen, 1904.
Reid. Todd's Cyclopaedia of Anatomy and Physiology. 1836–1839, **2**, 589.
Ribbert, Hugo. Handbuch der speziellen pathologischen Anatomie und Histologie, Henke, L., and Lubarsch, O. J. Springer, 1924, **2**, 184.
Ritter, S. A., Gross, L., and Kugel, M. A. Blood vessels in the valves of normal human hearts. From a study of 700 human hearts. *Am. Heart J.*, 1928, **3**, 433.
Seipp, L. Das elastische Gewebe des Herzens. *Anat. Hefte*, 1896, **6**, 63.
Stricker, S. Schweigger-Seidel's Manual of Human and Comparative Histology. Trans. Henry Power. London, 1870, **7**, 251.
Tandler, J. Anatomie des Herzens. Gustav Fischer, Jena, 1913.
Testut, J. L. Traité d'anatomie humaine. Paris, 1905, **2**, 80.
Torrigiani, C. A. Studio sullo sviluppo e sulla struttura dei seni del Valsalva e delle valvole semilunari nel cuore umano. *Arch. di anat. e di embriol.*, 1911, **9**, 570.
Veraguth, O. Untersuchungen über normale und entzündete Herzklappen. *Virchows Arch. f. path. Anat.*, 1895, **139**, 59.
Weber, A., and DeGuy, M. La région mitro-aortique. Étude anatomique et pathologique. *Arch. de méd. exper., et d'anat. path.*, 1897, **9**, 235–249, 339–378.
Zuckerkandl, E. Ueber Muskelgewebe in der Kammerfläche der Valvula tricuspidalis. *Allg. Wien. med. Ztg.*, 1877, **22**, No. 18, 155.

DESCRIPTION OF PLATES

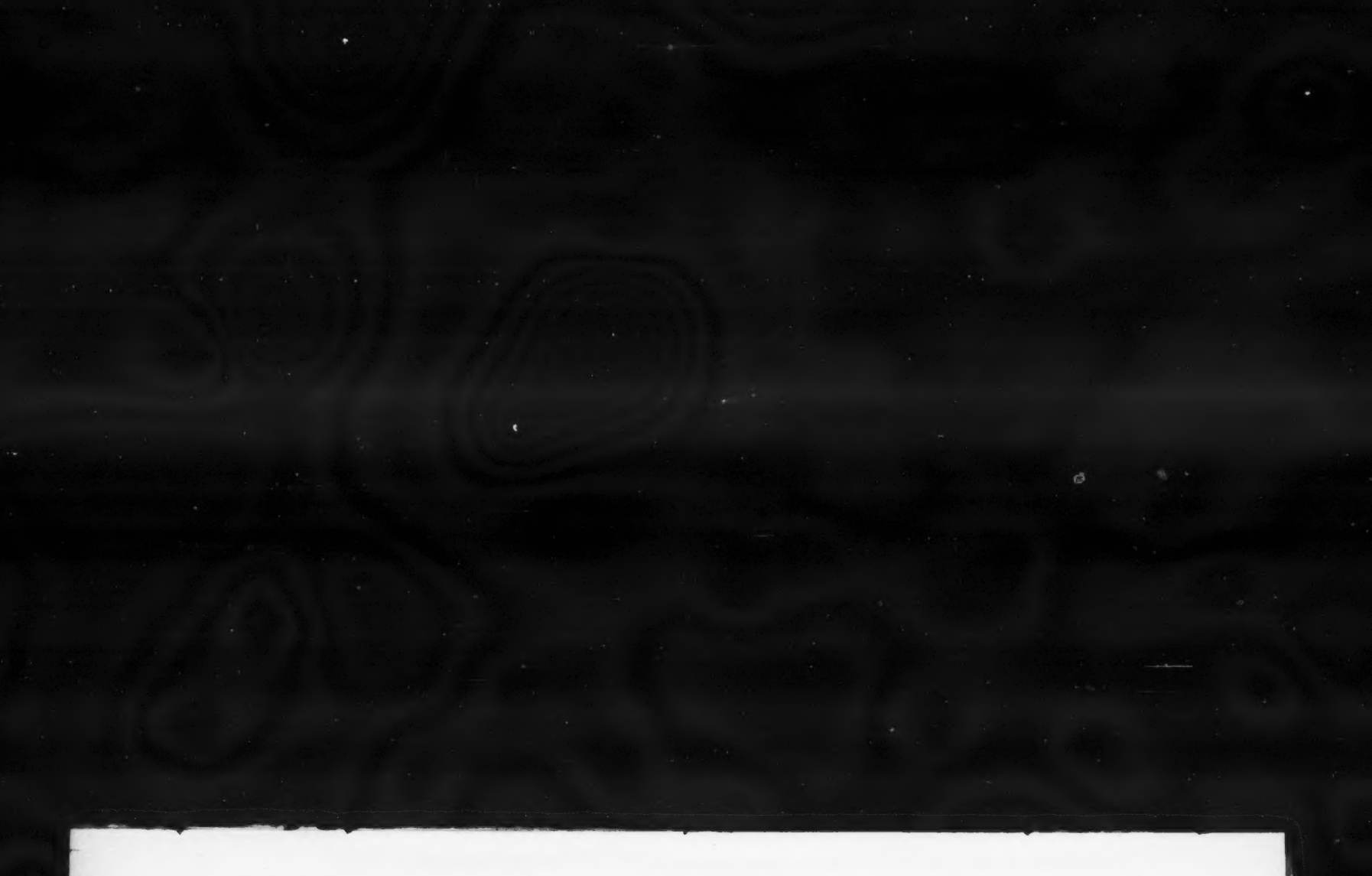
PLATE 102

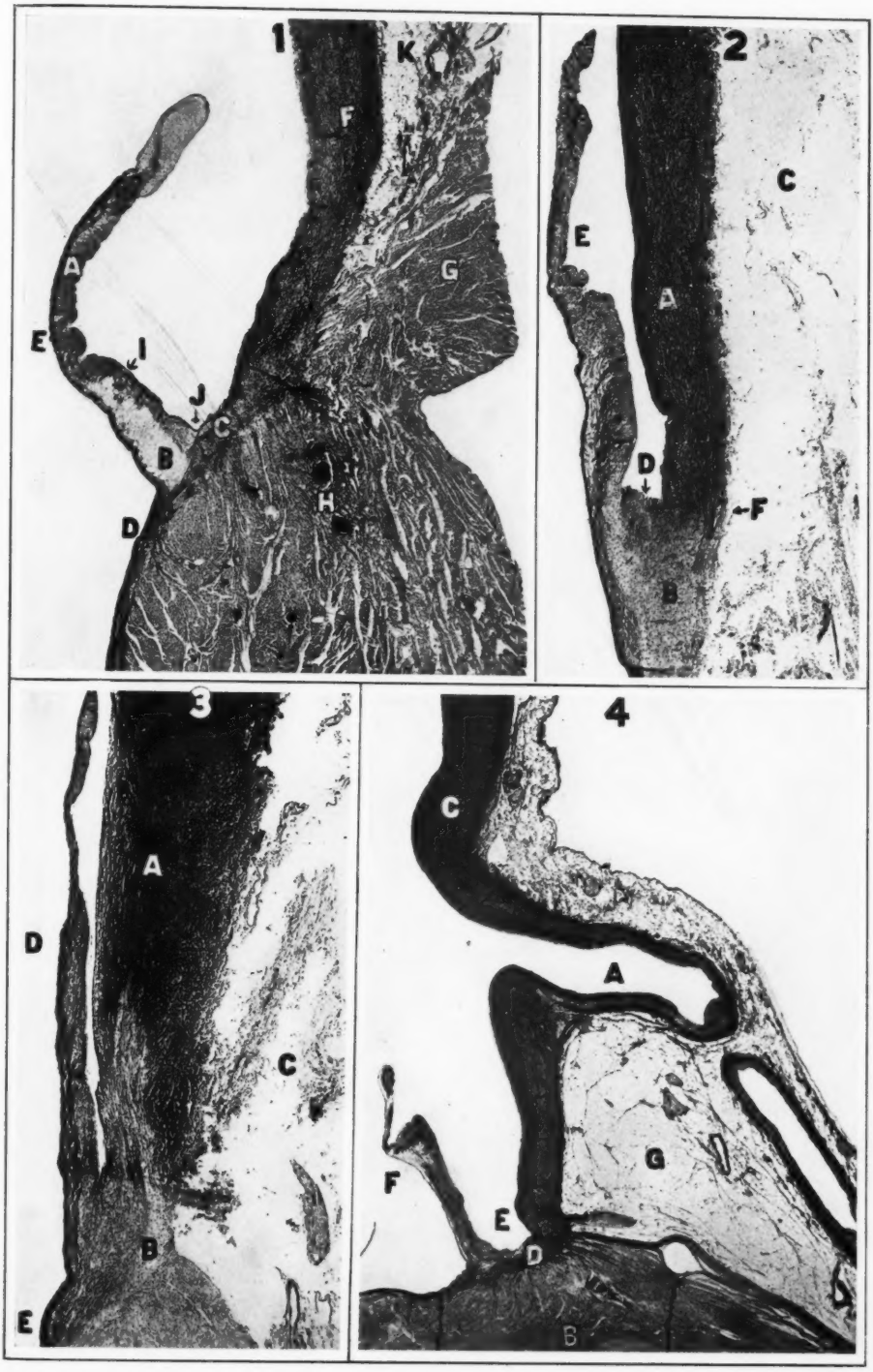
FIG. 1. *Right Aortic Cusp Section.* A, fibrosa; B, ring spongiosa; C, Annulus; D, subvalvular annulus; E, ventricularis; F, aorta; G, pulmonary conus; H, interventricular septum; I, arterialis; J, sinus pocket; K, pericardial wedge.

FIG. 2. *Aortic Cusp Approaching the Commissure* showing changes in the arterio-annulus relations. A, aorta; B, annulus; C, pericardium; D, sinus pocket; E, aortic cusp; F, arterio-annulus junction.

FIG. 3. *Aortic Cusp in the Immediate Vicinity of the Commissures* showing further changes in arterio-annulus relations. A, aorta; B, annulus; C, pericardium; D, aortic cusp; E, subcommissural elastica.

FIG. 4. *Left Aortic Cusp Section.* A, left coronary artery; B, left ventricle; C, aorta; D, annulus; E, sinus pocket; F, aortic cusp; G, free visceral pericardium.





Gross and Kugel

Anatomy and Histology of Valves in Heart

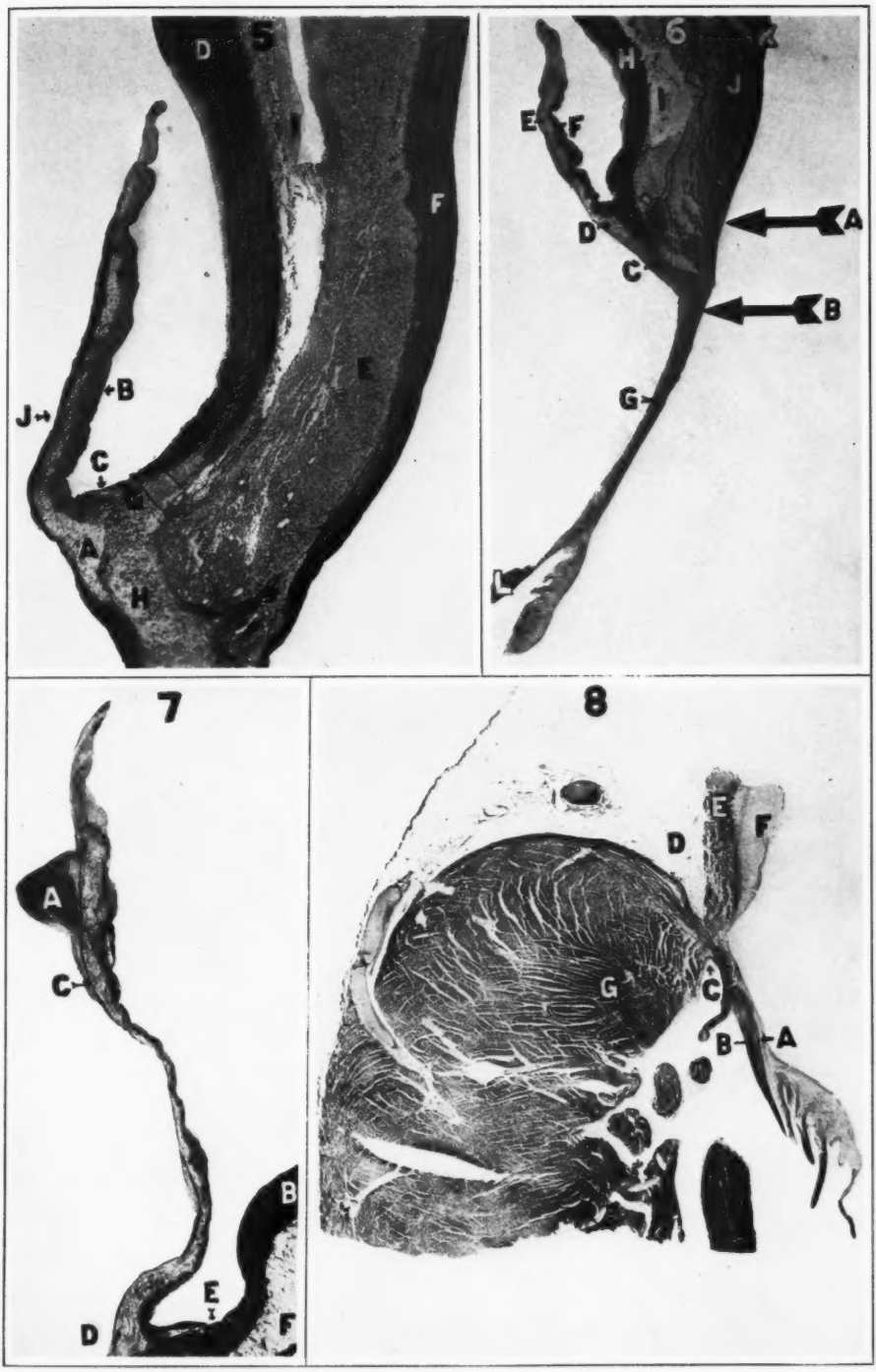
Plate 103

Fig. 5. *Posterior Aortic Cusp Section.* A, ring spongiosa; B, arterialis; C, sinus pocket; D, aorta; E, left auricular myocardium; F, left auricular endocardium; G, annulus; H, mitral-aortic intervalvular fibrosa; I, pericardial wedge; J, ventricularis.

Fig. 6. *Anterior Mitral and Posterior Aortic Valve Section.* For explanation of arrow "A" and arrow "B," see text. C, mitral-aortic intervalvular fibrosa; D, ring spongiosa; E, valve spongiosa; F, aortic valve fibrosa; G, mitral valve fibrosa; H, aorta; I, pericardial wedge; J, myocardial wedge (left auricle); K, left auricular endocardium; L, chorda tendinea.

Fig. 7. *Nodulus Arantii* on aortic cusp. A, nodulus arantii; B, aorta; C, ventricularis elastica; D, ring spongiosa; E, sinus pocket; F, pericardial wedge.

Fig. 8. *Posterior Mitral Valve Section.* A, spongiosa; B, fibrosa; C, posterior mitral valve pocket; D, pericardial wedge; E, auricular myocardial wedge; F, left auricular endocardium; G, left ventricle.



Gross and Kugel Anatomy and Histology of Valves in Heart

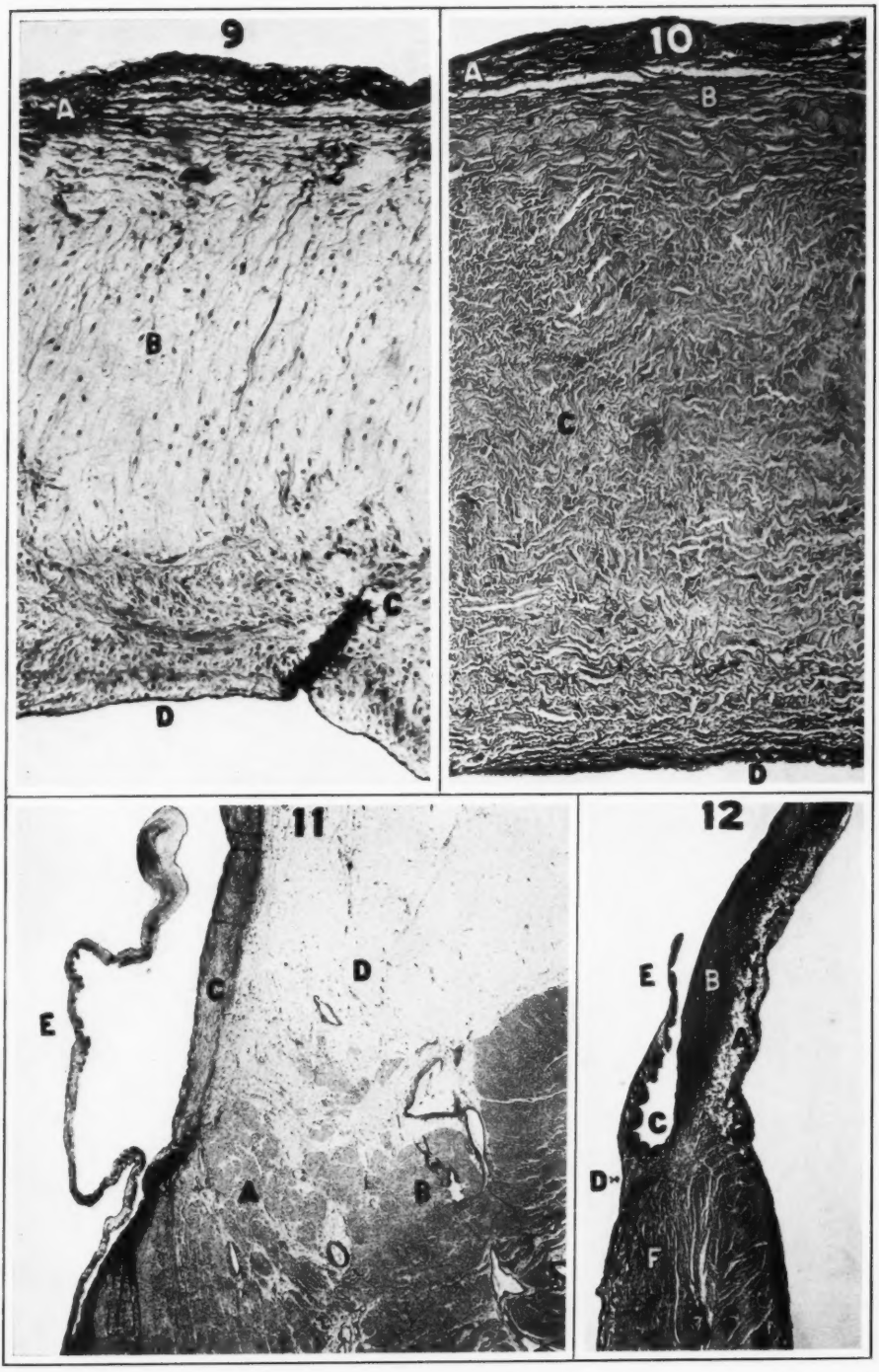
PLATE 104

FIG. 9. *Cross-section of a Semilunar Cusp* (High Power). A, ventricularis with its prominent elastica; B, spongiosa; C, fibrosa; D, arterialis.

FIG. 10. *Cross-section of an Auriculoventricular Cusp* (High Power). A, auricularis; B, spongiosa (inconspicuous); C, fibrosa; D, ventricularis.

FIG. 11. *Left Pulmonary Cusp Section.* Note double myocardial crest made up of A, right ventricle, and B, left ventricle. C, pulmonary artery; D, pericardium; E, pulmonary cusp.

FIG. 12. *Anterior Pulmonary Cusp Section.* A, pericardium; B, pulmonary artery; C, sinus pocket; D, ring spongiosa; E, pulmonary cusp; F, myocardium of pulmonary conus.



Gross and Kugel

Anatomy and Histology of Valves in Heart

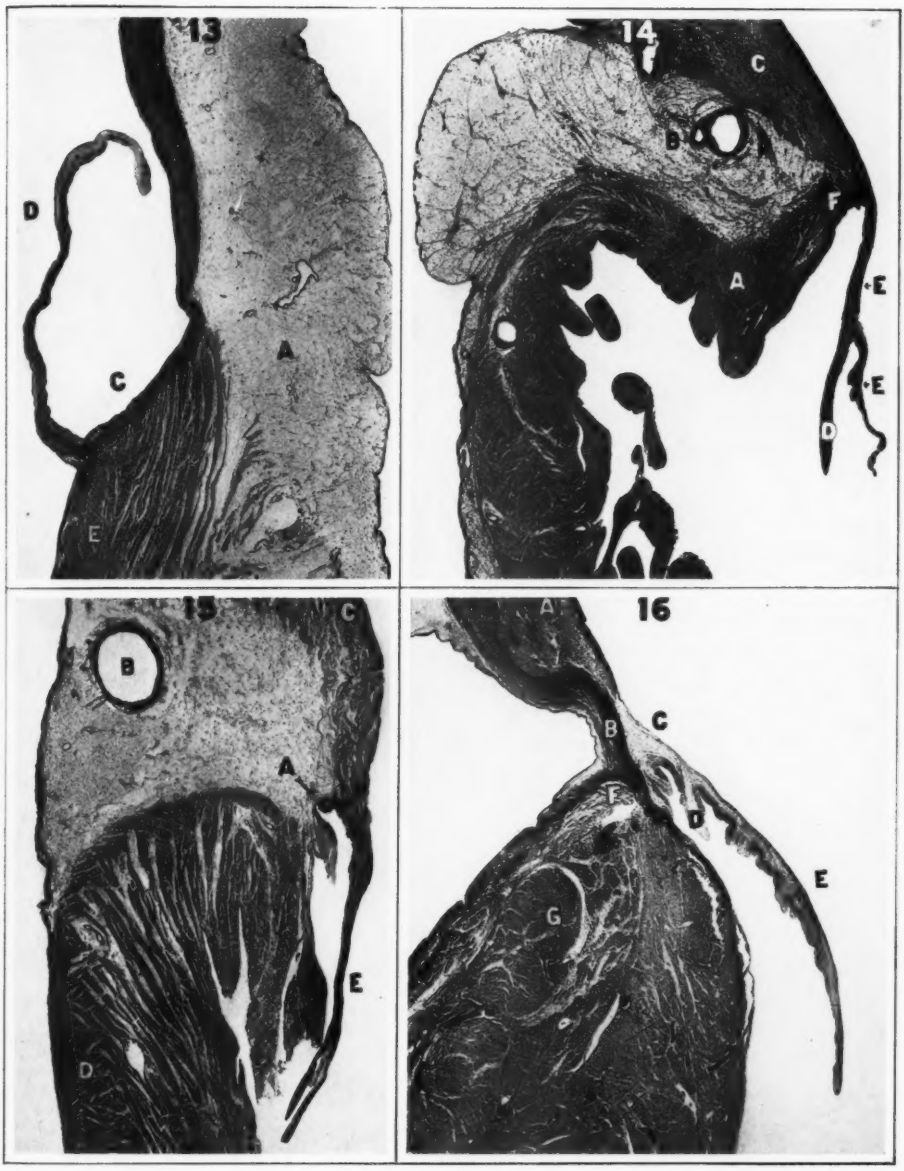
Plate 105

Fig. 13. *Right Pulmonary Cusp Section.* A, pericardium; B, pulmonary artery; C, sinus pocket; D, valve cusp; E, ventricular myocardium.

Fig. 14. *Anterior Tricuspid Valve Section.* A, trough-shaped myocardium of right ventricle; B, pericardial wedge with right circumflex coronary artery; C, right auricle; D, chorda tendinea; E, anterior tricuspid valve section; F, annulus.

Fig. 15. *Posterior Tricuspid Valve Section.* A, neck-like annulus; B, right coronary artery embedded in pericardium; C, right auricle; D, right ventricle; E, tricuspid leaflet.

Fig. 16. *Tricuspid Valve Section* (septal flap). A, right auricular myocardial wedge; B, septum fibrosum; C, tricuspid valve ring; D, chorda tendinea insertion; E, septal flap; F, bundle of His; G, interventricular septum.


13
D
C
A
E
14
C
B
F
A
E
E
D
15
C
B
A
E
D
16
A
B
C
F
D
E
G

AVIAN TUBERCULOSIS IN NORMAL AND VACCINATED RABBITS *

E. M. MEDLAR, M.D.

(From the Hegeman Memorial Research Laboratory of the Metropolitan Life Insurance Company Sanatorium, Mount McGregor, N. Y.)

The purpose of this report is to set forth the differences in the histopathology and in the leukocytic response in normal rabbits injected intravenously with the avian tubercle bacillus, and in rabbits which have been given some degree of protection by a previous subcutaneous inoculation of living tubercle bacilli of the human type. As far as we know, there is no record of spontaneous avian tuberculosis in the rabbit. Subcutaneous inoculation of virulent avian tubercle bacilli, even in doses of 10 mg., results in the formation of an abscess at the site of inoculation, with no generalized disease developing. With the rupture of the abscess this local lesion heals and the animal fully recovers.

If, however, one injects 0.5 to 1 mg. of virulent avian tubercle bacilli intravenously, rabbits will consistently die within a month. This is common knowledge to all who have used this method. The form of tuberculosis produced is commonly known as the Yersin septicemic type. It is frequently stated that there is no tubercle formation in the tissues in such animals. Blood cultures, taken at various intervals after infection, usually reveal the presence of the bacillus in the circulation.

AVIAN TUBERCULOSIS IN NORMAL RABBITS

The twenty normal rabbits used in this study were given an intravenous injection of 0.5 to 5 mg. of virulent avian tubercle bacilli and all died within a month.

The most striking features of the gross pathology were the marked enlargement of the spleen, and, at times, a moderate enlargement of the liver. Neither organ had gross tubercles, such as occur in rabbits inoculated with the bovine type of tubercle bacillus. The spleen

* Received for publication May 30, 1931.

was enlarged three to four times its normal size. The lungs were but slightly involved, and rarely had a discrete lesion which would suggest tubercle formation. The bone marrow showed no gross evidence of tubercle formation, but it was always firm and congested. None of the other organs gave any evidence of involvement on gross examination.

A study of the histopathology of the various organs of these animals showed a number of interesting things. On the whole, all of the animals presented the same type of reaction in the various tissues, the only variations being in degree and not in absolute difference. Because of such close similarity in distribution of lesions and type of reaction it is deemed unnecessary to give protocols on each animal. The histological description will deal with the changes observed in the group as a whole.

The lungs had numerous areas in which there was considerable thickening of the alveolar walls. This thickening was due largely to an accumulation of mononuclear leukocytes. There was an occasional lymphocyte, and in some of the areas a few neutrophiles were present. In these lesions tubercle bacilli were very scarce. The adjacent alveoli were often filled with an exudate similar to that present in the alveolar walls. On two occasions discrete lesions heavily infiltrated with neutrophiles and showing evidence of early caseation were found. Both of these lesions contained large numbers of tubercle bacilli. On numerous occasions typical mononuclear tubercles were observed within the veins. In such lesions tubercle bacilli were scarce, but could be demonstrated.

If a considerable amount of exudate was present in the alveolar spaces, giant cells of the type usually seen in tuberculosis could be found free within the alveolar spaces. On rare occasions a few tubercle bacilli could be demonstrated in these structures. Bacilli were always scarce in the exudate even when it was abundant. The cellular content consisted almost entirely of mononuclear leukocytes.

The heart muscle occasionally showed small collections of lymphocytes or of mononuclear leukocytes. No tubercle bacilli could be demonstrated.

No evidence of inflammatory reaction was seen in sections of the gastro-intestinal tract. Very rarely one found tubercle bacilli within single mononuclear leukocytes in the lymphoid tissue of the gut.

These animals commonly had diarrhea and we were surprised not to find tuberculous lesions.

The pancreas appeared normal in all sections studied.

The kidneys showed very little evidence of inflammatory reaction. Such foci as were found consisted of small collections of lymphocytes and an occasional mononuclear leukocyte. Bacilli could not be demonstrated. On rare occasions tubercle bacilli within a mononuclear leukocyte were observed in an otherwise normal glomerulus.

The adrenals usually had a few small collections of mononuclear leukocytes in the cortex. The medullary tissue always appeared normal. Bacilli were usually abundant in the lesions. A few foci composed largely of neutrophiles were seen. Tubercle bacilli were most abundant in such areas.

No lesions or bacilli were demonstrated in the genital organs of either the male or female rabbits.

No sections of joint tissue were studied, as all joints appeared normal in the gross.

Lesions in the bone marrow were a constant finding, as evidenced by discrete collections of mononuclear leukocytes. In some of the rabbits the major portion of the marrow was composed of a diffuse infiltration of these cells. Necrosis of the mononuclear leukocytes, with varying degrees of infiltration with neutrophiles, was often observed. Bacilli were easily demonstrated wherever the monocytic accumulation was found, and were present in large numbers in the more diffuse lesions. The hematopoietic tissue of the marrow was always hyperplastic even though extensive tuberculosis was present. Mitotic figures were easily found in leukopoietic and erythrogenic centers. Megakaryocytes were increased in number and mitoses were frequently found in cells of this type. These cells were often seen projecting into, and free within the capillaries of the marrow. A considerable number of megakaryocytes had neutrophiles within their cytoplasm. Foreign body giant cells of the type seen in tuberculosis were very rare, even when the lesions were extensive.

The liver always showed extensive involvement. In some instances the volume of inflammatory reaction was greater than the volume of liver tissue. The reaction consisted largely of mononuclear leukocytes within the capillaries of the organ. Occasional mitotic figures were found in the mononuclear leukocytes. The parenchyma of the liver was often markedly compressed, due to the extensive intra-

capillary accumulation of cells. Tubercle bacilli were present in large numbers.

One of the interesting findings in the liver was the presence of giant cells which had all of the characteristics of megakaryocytes. In some areas there would be three or more of these cells in a high power field. Judging from their frequency in sections from different parts of the organ, the livers of some of the animals must have contained a large number of these cells. They were not observed in sections of the liver in every animal.

The cytological reaction observed in the spleen in these rabbits was the most interesting observed in any of the organs. Engorgement of the organs with blood was always present. In the majority of instances the splenic corpuscles were very small. Many of the germinal centers could be identified only by locating the central arteriole. A marked increase of mononuclear leukocytes was a constant finding. In some instances this increase was diffuse throughout the organ. At other times the pulp was studded with mononuclear tubercles. Some of these tubercles, especially in animals living about four weeks, showed necrosis of the mononuclear leukocytes with slight to marked infiltration with neutrophiles. Typical caseation was seldom seen, while early tuberculous abscesses were often present. Foreign body giant cells were rarely encountered. Tubercle bacilli were always abundant. The lesions presenting necrosis of the mononuclear leukocytes with neutrophilic infiltration contained the greatest number of bacilli.

Large giant cells, similar to those seen in the liver, were very commonly found, at times as many as eight to ten in a high power field. These cells were observed in the germinal centers, in the splenic pulp and free in the venous sinuses. Mitotic figures were occasionally seen in these cells. In rare instances neutrophiles were seen within the cytoplasm of these giant cells, just as they were observed in the megakaryocytes in the bone marrow of the same rabbit.

The malphigian corpuscles of the spleen were so insignificant in the sections studied, as compared with the same structures in the normal rabbit, that it was deemed advisable to kill animals at different stages of the disease to study the changes that occurred in this organ. A complete report of these studies and their apparent significance will be given at a later date. The most significant finding was the

tremendous hyperplasia that occurred in the germinal centers up to about fourteen days after the injection. Because of the large numbers of mitoses and of the extension of the germinal centers well beyond their normal borders into the splenic pulp, one would be led to suspect that a malignant condition existed, were it not known that this reaction was due to infection with the tubercle bacillus.

The Leukocytic Response

The leukocytic response in these rabbits was determined by daily total and differential counts. The technique used was the same that we have adopted in the study of the human blood. Blood samples were obtained in all instances from a vein in the ear. Total counts were made from a 1: 20 dilution of the blood, using the Levy counting chamber. Blood smears were stained with Wright's blood stain. We recognize that in rabbits' blood it is difficult to distinguish clearly between young mononuclears or monocytes and large lymphocytes with this method of staining. However, as the disease process advanced, the change in the percentage of the different leukocytic types was so marked that one could gain a fairly accurate idea of the pathological process as a whole with this technique. We have found this procedure to be simpler and less time-consuming than the supravital method of staining.

To illustrate the changes in the circulating leukocytes during the evolution of the disease, charts R-Bl., and R-8 have been included. These show the variation that may occur in different animals with different doses of bacilli. We found it impossible to get a satisfactory average leukocytic picture in normal rabbits, so we adopted the procedure of using each animal as its own control by making daily counts for a week or longer before the injection of tubercle bacilli was given.

Only the main features in the change of the leukocytic picture will be discussed as all the fluctuations are not considered significant. The variations are largely individual changes that one would expect to occur in different animals. Two rabbits in the series died in fourteen days. Both of these animals were given an injection of 5 mg. of avian tubercle bacilli and each developed a marked leukopenia with the greatest decrease occurring in the lymphocytes. At the time of death the lymphocytes were less than 1,000 per cubic millimeter.

The neutrophiles and mononuclear leukocytes were about equal in number and were more than 1,000 per cubic millimeter.

The leukocytic response in all the other animals was fairly constant in the differential percentages, but showed considerable variation in the total leukocytic counts. Twenty-four hours after the injection of the tubercle bacilli a slight rise in neutrophiles occurred. At the same time, or more often at the end of forty-eight hours, an initial small rise in mononuclear leukocytes could be demonstrated. This initial response on the part of the neutrophiles and mononuclears receded promptly to normal for the individual rabbit. At this stage no significant change in the lymphocyte count was manifest.

For a period of eight to ten days the most striking change in the leukocytes was a tendency for the neutrophiles to decrease in number and for a slight leukopenia to develop. After this stage of incubation a rapid alteration in the leukocytic formula occurred which persisted until death. The first outstanding change was a rapid rise in the number of mononuclear leukocytes. The peak of this rise was often reached within three or four days after its inception, and from this time on these cells did not recede to a normal level. Great daily fluctuations in counts were observed. During this stage one occasionally encountered mononuclear leukocytes undergoing mitosis. The cells were markedly vacuolated at times and it was not uncommon to find red blood cells within their cytoplasm.

Prior to the increase of circulating mononuclear leukocytes there was no significant change in the number of lymphocytes. Some of the animals showed a sharp decrease in the number of lymphocytes at the time of the main mononuclear response. From the date of the most marked increase of mononuclear leukocytes to the death of the animal, the number of circulating lymphocytes tended to decrease, although one observed a considerable fluctuation from day to day. In several rabbits these cells decreased to less than 500 per cubic millimeter, whereas the same rabbits had 5,000 to 8,000 lymphocytes per cubic millimeter before they were infected.

The neutrophilic response in these rabbits usually lagged behind that of the mononuclear leukocyte. In every rabbit the neutrophiles returned to a normal level within two or three days after the mononuclear rise. From this time on these cells tended to be above normal and they were generally the predominant cell type before the

animals succumbed to the infection. Here again considerable daily fluctuation was observed.

There were marked variations in the numbers of basophiles and of eosinophiles during the progress of the disease, but toward the end of the process these cells were scarce in the circulating blood.

The total number of leukocytes showed considerable variation from day to day. A mild leukocytosis was observed as the disease progressed and in some of the animals there occurred a marked leukocytosis before death.

Red blood cell counts were not done as these cells take no part in the production of the tuberculous lesions. It was noted, however, that there was marked anemia and at times nucleated red blood cells were numerous in the blood films.

Blood platelet counts were not done.

Avian Tuberculosis in Vaccinated Rabbits

We turn now to the rabbits which were partially protected. There were but four rabbits in this group. While the group is small the findings differ so markedly from those in the non-vaccinated group that we consider them very significant. As daily blood counts were done on this group and on a group of eight normal rabbits inoculated with the same dose of avian tubercle bacilli on the same date, we felt that the work entailed prevented a larger group from being studied. We felt also that intensive work done on a few animals would give more significant data than less work on a larger group.

These four rabbits were originally used in another study. A subcutaneous inoculation of 5 mg. of human tubercle bacilli, H-37, had been given six months before they were used in the present experiment. Each animal had developed a local abscess at the site of inoculation. These abscesses had ruptured and the local lesions had healed. At the time of inoculation with the avian tubercle bacillus one rabbit showed a small lump about the size of a pea at the site of the previous inoculation. The other rabbits showed no local lesions. These findings were confirmed later at autopsy. When inoculated with the avian bacillus the rabbits were in excellent physical condition and their blood counts were normal.

The group of twelve rabbits, eight normal and four vaccinated, were given an intravenous inoculation of 1 mg. of virulent avian

tubercle bacilli. The non-vaccinated animals all died within 24 days. In the vaccinated group one animal died on the 149th day, one on the 140th day, one on the 122d day, and one on the 7th day.

The last rabbit succumbed so quickly that we at first thought it died of some intercurrent infection. At autopsy the spleen was found markedly enlarged, the liver was slightly enlarged, and there was no evidence of lobar pneumonia. There was no evidence of snuffles. No typical tubercles were seen in any organ on gross inspection. Joints, kidneys, testes and epididymes appeared normal. The bone marrow of the femur was firm and red. At the site of the inoculation with human bacilli there was a small scar. The gross findings were identical with those of normal rabbits inoculated with the avian tubercle bacillus, except that no normal rabbit had ever shown such marked enlargement of the spleen and liver at such an early date. The absence of old tubercles in any of the organs is of importance in consideration of the lesions the other rabbits in the group showed.

Histological studies of the tissues of this rabbit showed extensive tubercle formation in the spleen, liver and bone marrow. Pulmonary lesions similar to those described above for the normal rabbit group were present. A finding of some surprise was the extensive tubercle formation and the scarcity of tubercle bacilli. Bacilli were found but they were very infrequent. Foreign body giant cells were much more numerous than in the lesions of the non-vaccinated rabbits.

Cultures were not made of the lesions of this animal, but from the extensive pathology in the liver, spleen and bone marrow we feel convinced that death was due to the avian tubercle injection given a week before.

The pathology of the other three rabbits in the group was so uniform that we will describe them as a group. All of the rabbits before death became so emaciated that they could not stand. Their appetites remained good and they would eat food if it were placed where they could reach it in the prone position.

The gross pathology showed a striking contrast to that seen in the non-vaccinated animals. The spleen was of normal size and of a dark brown color. Rare firm, grayish tubercles of millet-seed size were present. The liver was of normal size. There were numerous scars and occasional minute tubercles were present beneath the capsule and in the liver substance. The lungs in one rabbit were normal. In each of the other two there were found two or more foci

of typical tuberculosis. These areas appeared like conglomerate tubercles. The gastro-intestinal tract was negative throughout. There was extensive bilateral renal tuberculosis. The adrenals appeared normal. Two of the rabbits showed circumscribed tuberculous lesions in the testes and epididymes. The bone marrow was congested and friable but no gross tubercles were seen. Each of the rabbits showed multiple tuberculous joints, with abscess development of considerable extent at some of the joints, notably knees, hips and elbows. Some of these lesions extended for a considerable distance along the fascial planes of the muscles. At the site of inoculation of the human tubercle bacillus two rabbits showed small scars. The third rabbit showed a small caseous lesion. This lesion had enlarged and had discharged its contents during the course of the avian infection.

Smears from the various tuberculous lesions showed a few to many acid-fast bacilli. No other bacteria were seen. Cultures from the abscessing joints and from the kidneys gave a pure growth of acid-fast bacilli of the avian type. No human type of tubercle bacilli were obtained in these cultures. From the bacteriological point of view it would seem that, although the lesions differed in gross appearance from those seen in the unvaccinated animals, they were caused by the avian tubercle bacillus.

Smears made from the residual caseous lesion of the human tubercle bacillus infection present in one of the rabbits revealed no acid-fast organisms and culture was negative.

Histological studies of the tissues of the three animals revealed typical tubercle formation with caseation and numerous giant cells. There was calcification in many of the lesions. The bone marrow revealed an occasional foreign body giant cell and a rare tubercle composed largely of scar tissue and of lymphoid cells. There was marked atrophy of the fat tissue. The hematopoietic tissue was moderately increased. Mitotic figures were easily found, especially in the leukopoietic centers. Megakaryocytes were not greatly increased in number. Many of these cells had neutrophiles within their cytoplasm. Bacilli could not be demonstrated in the marrow lesions.

In the splenic pulp there was a very heavy deposit of brownish pigment. The germinal centers appeared smaller than normal but mitotic figures were occasionally seen. Tubercles were scarce and

those that were present showed slight caseation, foreign body cells, considerable lymphocytic infiltration and fibrosis. Isolated foreign body giant cells were commonly seen. Bacilli were found with difficulty.

The major portion of the liver tissue appeared normal. There were occasional tubercles with central caseous areas, giant cells, and considerable lymphoid infiltration in the periphery. Considerable fibrosis was present in most of the lesions. There were many small collections of lymphoid cells in the parenchyma. These evidently were the remains of a once active tuberculous focus. Microscopic abscesses were occasionally seen. Scars, composed of fibrous tissue infiltrated with lymphocytes, were present. These probably represented healed tuberculous lesions. Many isolated foreign body giant cells were seen. Bacilli were not found in the scarred areas or in the collections of lymphoid cells. They were found after long search in some of the caseous tubercles, and with ease in the abscesses.

The kidneys showed large, caseating tuberculous lesions, most of which were pyramidal in shape, extending from the cortex into the pyramids. Giant cells were rare. Occasional scars were present. In some lesions, where neutrophiles were abundant, bacilli occurred in large numbers. In the old caseous lesions bacilli were very scarce and in the scars bacilli could not be demonstrated.

Sections from the adrenals and the gastro-intestinal tract revealed no lesions. Sections from the lung lesions showed old caseous tubercles with some calcification and with rare bacilli present. In the lymph nodes there were a few small mononuclear tubercles. The testes and epididymes had caseous foci. Sections of tissue from around the joints revealed abscesses with caseating peripheries. Bacilli were easily found.

The Leukocytic Response

The leukocytic response of these animals was quite in contrast to the non-vaccinated group. Chart R-I-H-A, of the rabbit dying in 7 days, and Chart R-4-H-A of the one dying in 144 days are appended to give a graphic picture of what occurred. All of the animals had normal leukocyte counts prior to the inoculation of the avian bacillus, showing that they had recovered from their infection with the human bacillus. Chart R-I-H-A shows that the leukocytic picture

changed rapidly to a markedly septic type, and the animal quickly succumbed.

Chart R-4-H-A is typical of the three remaining rabbits. The mononuclear leukocytes played a prominent, but not so spectacular a part, as in the non-vaccinated animals. Considerable daily fluctuations occurred. For a considerable period before death these cells became as numerous or more numerous than the lymphocytes.

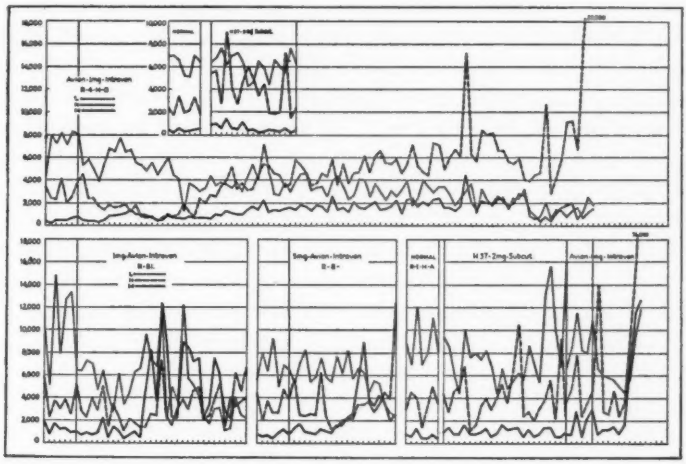


CHART 1

The most striking feature of the chart is the gradual change of the leukocytic picture to the septic type which remained, after it was once established, until the animal died. The neutrophiles also showed wide daily fluctuations. In each of the animals in this group the septic leukocytic reaction became definitely established weeks before the death of the animal.

The lymphocytic picture was just the reverse of the neutrophiles. There occurred a gradual decrease of these cells to a low level where they stayed, except for some daily fluctuation.

DISCUSSION

We have given above the pathology and the leukocytic reaction in normal and in vaccinated rabbits intravenously infected with virulent avian tubercle bacilli. While we used the avian tubercle bacillus as

our infective agent, we do not feel that the histopathology which it induced is significantly different from that produced by other types of tubercle bacilli.

There is no question that there may be a difference of degree in the histopathology caused by tubercle bacilli of the avian, bovine or human type, but there is no specific difference. One can find a great variation in the histopathology caused by bovine tubercle bacilli of varying degrees of virulence, or caused by a single strain of bovine tubercle bacillus given in different doses. It is quite probable that there is some difference in the chemical composition of the various types of tubercle bacilli. It is also probable that the chemical composition of a very virulent bovine tubercle bacillus differs somewhat from that of a bovine tubercle bacillus of low virulence. Specific biochemical reactions must occur in the tuberculous lesions but, at present, the histopathology seen cannot be interpreted on the underlying specific biochemical changes. The writer is of the opinion that if one takes into account the histopathological picture present in all the tissues it is impossible to state with certainty the type of tubercle bacillus that has been the infective agent.

There may also be a difference in the gross pathology of rabbits infected with the avian, bovine or human type of the tubercle bacillus. One cannot, however, regard this difference as specific. Our study shows that by altering the condition of the rabbit, namely by vaccinating it, the gross pathology caused by the avian tubercle bacillus differs markedly from the gross pathology observed in the non-vaccinated rabbit.

In all articles with which the author is acquainted, evidence of resistance in vaccinated animals is measured by the length of life, as compared to non-vaccinated controls, and the presence and the extent of gross tuberculous lesions. We wish to call attention to the fact that there is quite a striking difference in the histopathology in vaccinated and non-vaccinated animals. In the first place, tubercle bacilli are uniformly much more numerous in the lesions of non-vaccinated animals than in the vaccinated. Typical tubercles with caseous centers, giant cells, lymphoid infiltration and fibrosis are commonly seen in vaccinated animals, whereas such structures are not found in non-vaccinated animals when they die in from three to four weeks. Scars, often of considerable size, are found in the tissues of vaccinated animals, whereas they are not found in the non-

vaccinated. Organs which were very heavily involved in non-vaccinated animals gave little evidence of involvement or much permanent impairment in vaccinated animals. Organs which were very slightly involved in normal animals became, in the vaccinated, the seat of serious involvement, with some evidence of resistance as shown by the presence of fibrosis, calcification and giant cell formation. This resistance, however, was insufficient to overcome the massive damage done to such tissues. These findings are all the more significant, because in our experiments we have used an intravenous dosage of virulent avian tubercle bacilli that has in every instance killed normal rabbits within a month.

The findings given above help one to understand better the lesions one sees in chronic tuberculosis in animals and in human beings. In unreported experiments on prophylactic vaccination against tuberculosis in laboratory animals, we have commonly seen a marked difference in the histopathology between vaccinated animals and controls, but we had not appreciated that this difference signified the increased resistance on the part of the vaccinated animals. Failure to find tubercle bacilli in lesions with giant cells, lymphoid infiltration and fibrosis was attributed to non-stainable bacilli. We now believe that in the majority of such lesions the bacilli have been destroyed. This same interpretation would apply logically to the usual text book "epithelioid" tubercle in human tuberculosis and would explain the scarcity of tubercle bacilli in these lesions. In other words, tubercles with caseous centers and giant cells represent an increased resistance on the part of the host. If the pathology of tuberculosis were limited to this picture, one would not find cavitation and extensive involvement of tissues which lead to death. Such tubercles indicate a regression rather than a progression of the disease.

A considerable revival of interest has been created in the leukocyte reaction in tuberculosis of late. The pioneer work in this country, with regard to the new interpretation of the leukocytic reaction, is that of Sabin and her coworkers.[1] Their interpretation is based largely on the textbook description of the pathology of tuberculosis, in that they relegate the neutrophile to a place of no importance, so far as the tuberculous process *per se* is concerned. More recently we have offered [2] an interpretation of the leukocytic reaction based on a somewhat different interpretation of the pathology of tuberculosis.[3]

The striking difference in the leukocytic reaction of the two groups can be noted in the leukocytic graphs. The most striking feature is that the leukocytic picture gradually changed in the vaccinated rabbits to a septic reaction and remained so until the death of the animal. We do not attempt to explain this change. It is of interest, however, that in progressive cases of human tuberculosis the leukocytic reaction is very similar to that seen in the vaccinated rabbits. We are of the opinion that this septic reaction is in response to the damaged tissue in the tuberculous lesions and is due to unmixed tuberculous infection.

The vaccinated animal which died in 7 days was of interest. We have not had a normal animal succumb so quickly to a tuberculous infection, even when massive injections have been made. With such extensive pathology the scarcity of demonstrable tubercle bacilli was wholly unexpected. It seems probable that the previous vaccination with the human tubercle bacillus had enabled this animal to destroy the majority of the tubercle bacilli injected quickly, but the increased resistance was insufficient to render harmless the products of the destroyed bacilli. The flooding of the body with the toxic substances of the destroyed bacilli was probably so great that the animal was unable to render them harmless before death ensued. Even the quick and extensive response on the part of the leukocytes failed to stem the tide. One might postulate that the degree of resistance which the vaccination had produced was the prime factor in the sudden death of the animal. Whether the degree of resistance in this animal was greater or less than in the other animals in the group is an open question.

A renewal of interest in the relationship of Hodgkin's disease to tuberculosis has recently been created through the work of L'Esperance.[4] This article is of interest in relation to the reports of L'Esperance, as it presents the acute and chronic types of pathological reaction in the rabbit to the avian tubercle bacillus, which L'Esperance is inclined to believe is the etiological agent of Hodgkin's disease. There are certain phases of the pathology which simulate Hodgkin's disease. The significance of this observation will be reported in another article.

Summary and Conclusions

1. There is a significant difference in the gross pathology and in the histopathology of non-vaccinated and vaccinated rabbits intravenously inoculated with virulent avian tubercle bacilli.

2. The text-book description of the tubercle — "a collection of epithelioid cells set in a reticulum with a giant cell in the center and a tendency to undergo caseation" — represents a retrogressive, not a progressive phase of the pathology of tuberculosis.

3. There is a striking difference in the leukocytic reaction of non-vaccinated and vaccinated rabbits intravenously infected with virulent avian tubercle bacilli.

4. The leukocytic response in the vaccinated rabbits simulates very closely the leukocytic reaction we have observed in human beings who have progressive tuberculosis. This reaction is not specific for tuberculosis, but is caused by unmixed tubercle bacillus infection.

5. The megakaryocyte plays an important rôle in acute avian tuberculosis in the rabbit. What the real significance of the participation of the megakaryocyte in acute tuberculosis is, cannot be stated at present.

REFERENCES

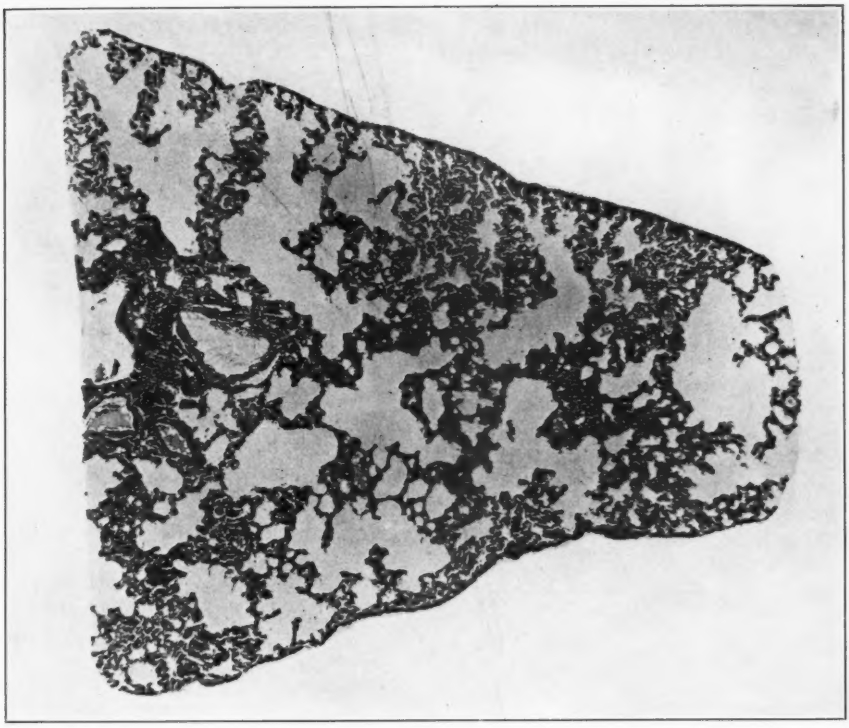
1. Cunningham, R. S., Sabin, F. R., Sugiyama, S., and Kindwall, J. A. *Bull. Johns Hopkins Hosp.*, 1925, **37**, 231.
2. Medlar, E. M. *Am. Rev. Tuberc.*, 1929, **20**, 312.
3. Medlar, E. M. *Am. J. Path.*, 1926, **2**, 275.
4. L'Esperance, E. S. *J. Immunol.*, 1930, **18**, 127.

DESCRIPTION OF PLATES

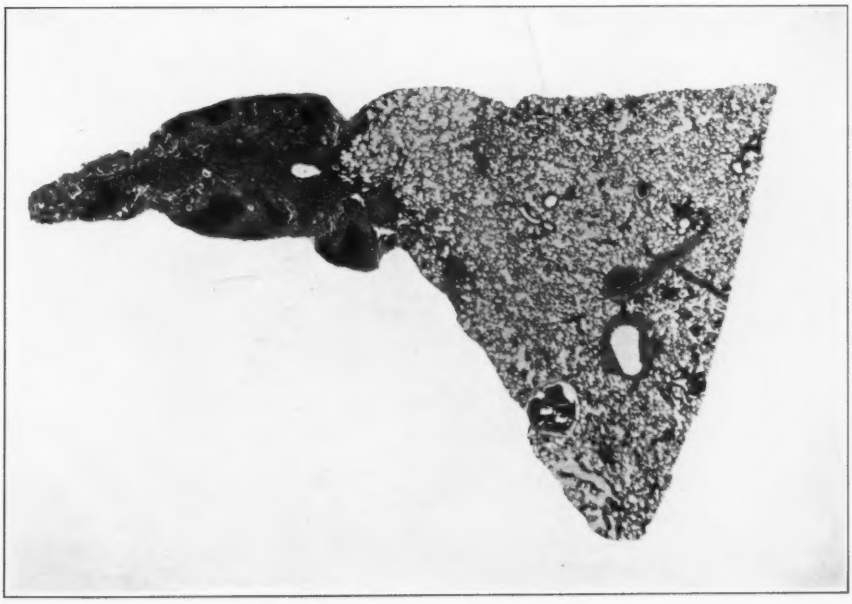
PLATE 106

FIG. 1. Lung from non-vaccinated rabbit. Numerous small tubercles. Death in 24 days. × 50.

FIG. 2. Lung from vaccinated rabbit infected with avian tubercle bacilli. Death in 140 days. × 10.



1



2

Plate 107

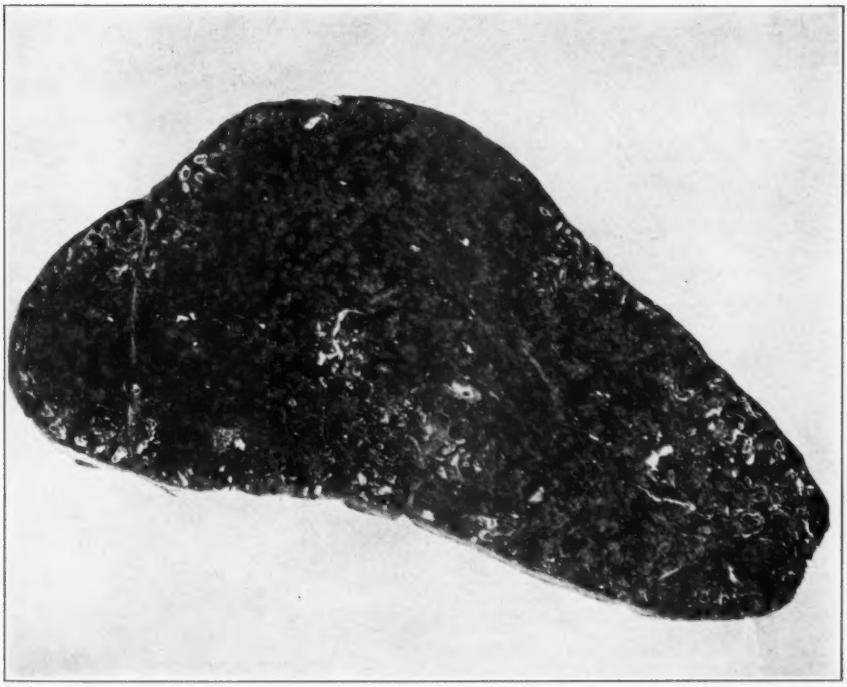
Fig. 3. Lung from non-vaccinated rabbit. Tubercle within vein. × 600.

Fig. 4. Spleen from vaccinated rabbit. Avian tubercle bacillus infection. Death in 140 days. × 10.

Fig. 5. Spleen from non-vaccinated rabbit. Death in 28 days. × 10.

3

4

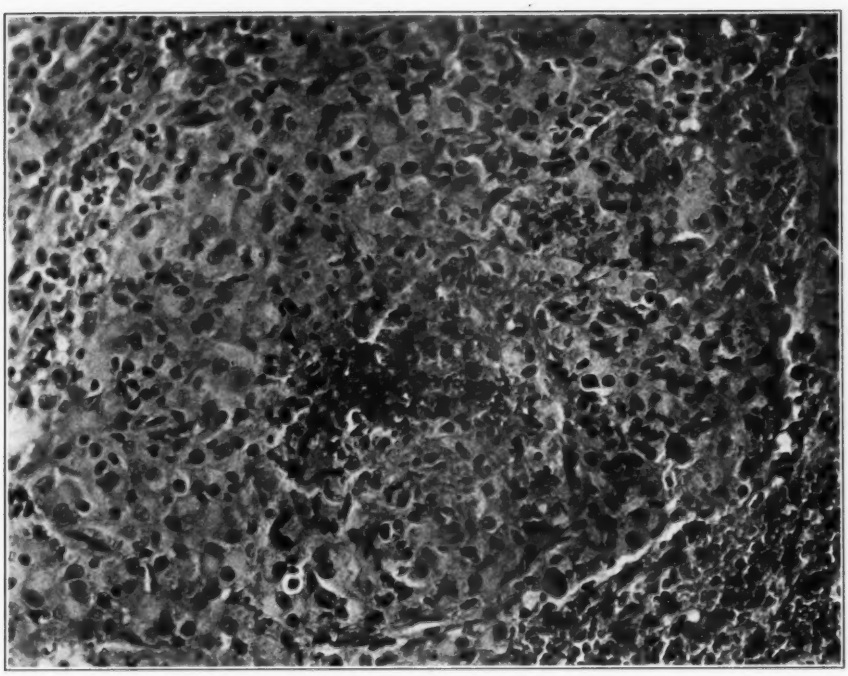


5

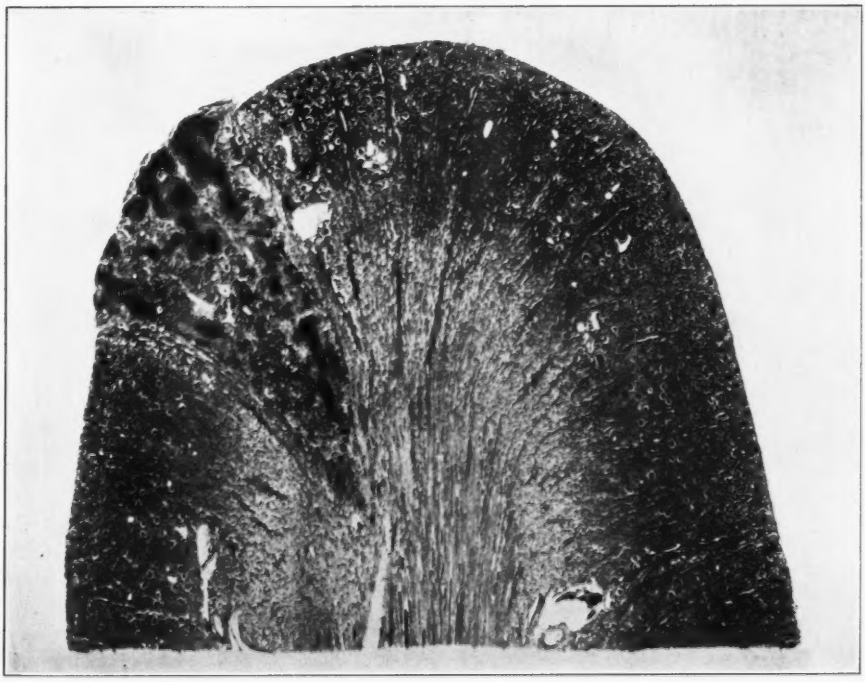
PLATE 108

FIG. 6. Spleen from non-vaccinated rabbit. Enlarged area of Fig. 5. Tubercle with neutrophile infiltration in center. × 500.

FIG. 7. Kidney from vaccinated rabbit. Death in 140 days. × 10.



6

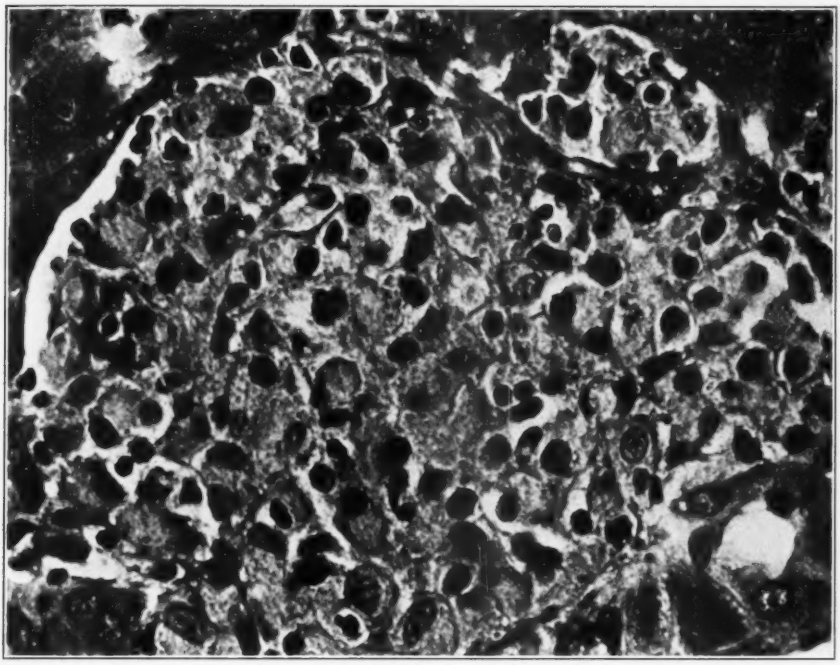


7

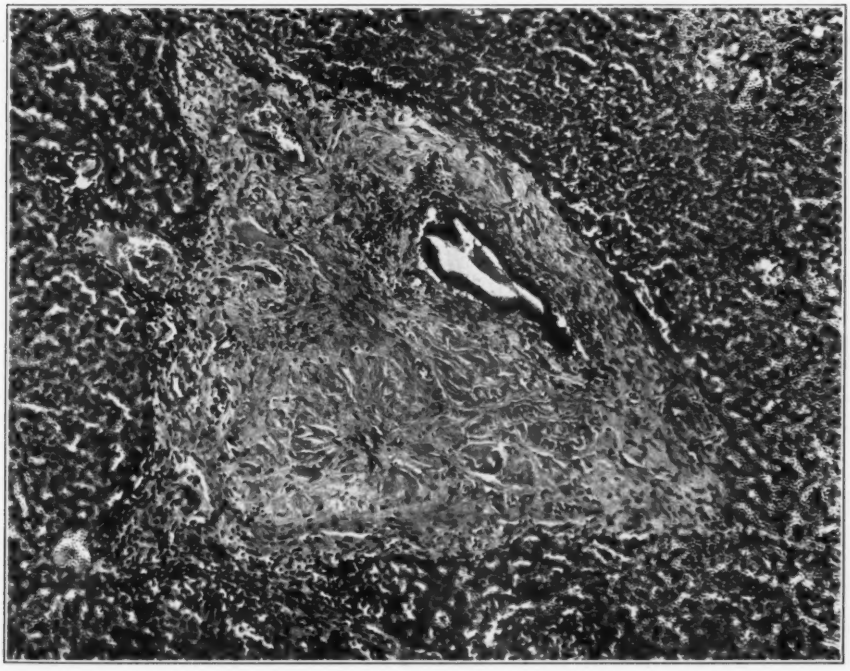
PLATE 109

FIG. 8. Liver from non-vaccinated rabbit. Tubercle in distended sinusoid. × 800.

FIG. 9. Liver from vaccinated rabbit. Large scar. × 10.



8



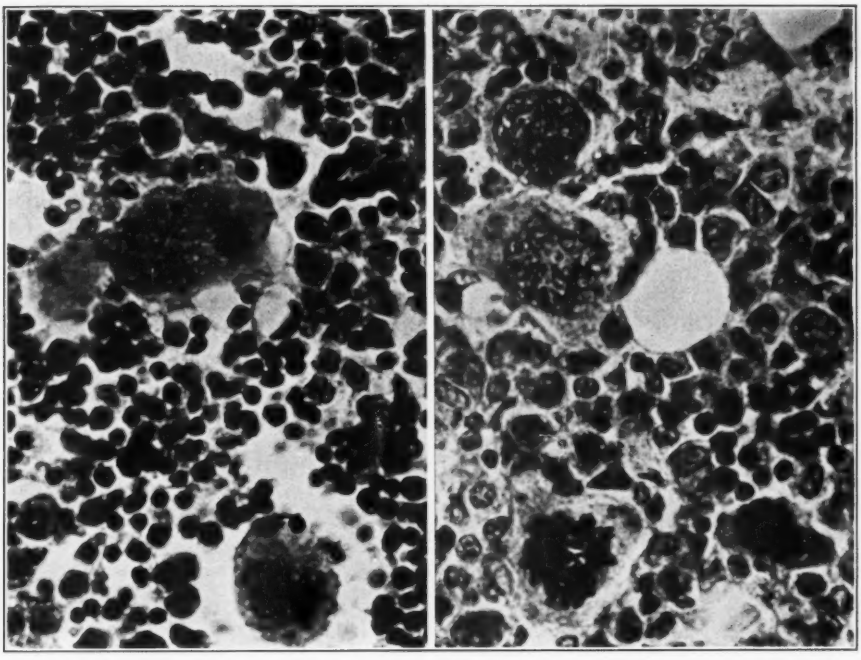
9

PLATE 110

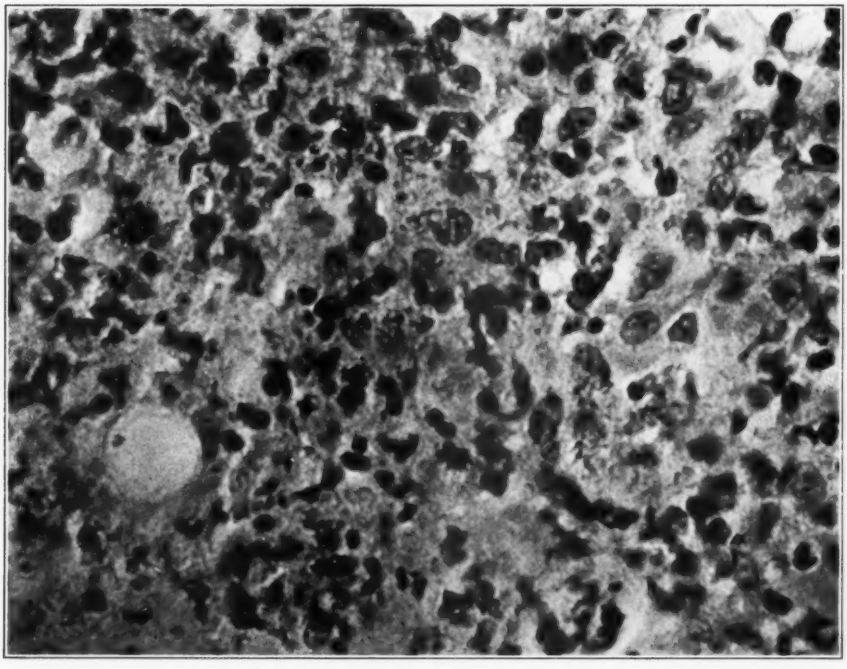
FIG. 10. Bone marrow from non-vaccinated rabbit. Lower megakaryocyte entering small vein to left. × 800.

FIG. 11. Same section as Fig. 10. Two megakaryocytes in mitosis. × 800.

FIG. 12. Marrow from non-vaccinated rabbit. This marrow showed extensive tuberculosis and large numbers of bacilli. Note bacilli, mononuclear leukocyte infiltration and some neutrophile infiltration. × 800.



10 11



12

Medlar Avian Tuberculosis in Rabbits

THE SIGNIFICANCE OF LESIONS RESEMBLING HODGKIN'S DISEASE IN TUBERCULOSIS *

E. M. MEDLAR, M.D., AND K. T. SASANO, M.D.

(From the Hegeman Memorial Research Laboratory of the Metropolitan Life Insurance Company Sanatorium, Mount McGregor, N. Y.)

Interest in the relationship between tuberculosis and Hodgkin's disease has been revived through the reports of L'Esperance.[1] According to these reports, L'Esperance is of the opinion that the tubercle bacilli which she obtained from certain cases of Pel-Ebstein disease were of the avian type. The deduction from her studies was that the avian tubercle bacillus probably has an etiological relationship to Hodgkin's disease.

Numerous investigators have reported on the association of tuberculosis and Hodgkin's disease. Ewing [2] states that "tuberculosis follows Hodgkin's disease like a shadow." While there have been numerous observations of the association of these two diseases no one has been able to reproduce in experimental animals the gross and histological pathology of Hodgkin's disease with the tubercle bacillus. Failure to reproduce Hodgkin's disease experimentally has led those who believe that the tubercle bacillus is the etiological factor to postulate that it is necessary to have a tubercle bacillus of a certain type and of a certain pathogenicity. To date this special type of organism has not been obtained.

Because of inconstancy of findings relative to the tubercle bacillus in Hodgkin's lesions other investigators have been led to search for a different infectious agent. The bacteriological studies of Bunting and Yates [3] revealed the common occurrence of a diphtheroid organism. With this organism they were unable to reproduce typical Hodgkin's disease experimentally. Other investigators have obtained still different bacteriological findings. Recently Haythorn [4] has obtained a culture of a monilia in a case of Hodgkin's disease. The varied bacteriological findings have led some who believe that Hodgkin's disease is of an infectious nature to the conclusion that the etiological agent has not been discovered.

* Received for publication May 30, 1931.

In this report we will present an interpretation of certain lesions resembling Hodgkin's disease which we have observed in our studies of the tissues in experimental tuberculosis, as well as similar lesions in a human being who died from extensive pulmonary tuberculosis.

Our first observations were on rabbits that had been infected intravenously with virulent avian tubercle bacilli. These animals died in from two to four weeks. The gross and microscopic pathology, as a whole, is reported in another article so that in this report we will mention only lesions simulating Hodgkin's disease. These animals did not give the gross pathology of Hodgkin's disease. Microscopic examination of the various tissues showed a few to many giant cells which were indistinguishable from the giant cells seen in Hodgkin's lesions. Such lesions were found in the lung, liver and spleen. The bone marrow in these animals was markedly hyperplastic and showed a marked increase of megakaryocytes. We were able to find the megakaryocytes in the process of entering the circulation and present within the blood sinuses of the marrow. These findings led us to the conclusion that the giant cells observed in the tissues were megakaryocytes.

In the hyperplastic marrows complex mitotic figures in the megakaryocytes were occasionally encountered. These, also, were found rarely in the spleen and liver. Such figures resembled very closely the complex mitotic figures occasionally seen in the giant cells of Hodgkin's lesions.

Mitoses were more commonly encountered in a cell type in the marrow that was much smaller than the megakaryocyte but was somewhat larger than the myelocytes in the same section. It appeared that these smaller cells were the parent cells or the pre-megakaryocytes. The significance of this observation will be dealt with in a subsequent report on Hodgkin's disease.

These lesions were much more numerous in some of the rabbits than in others. In animals that were given a smaller dosage of bacilli or were protected to a degree by vaccination with tubercle bacilli and lived for several months, not only was the type of the tuberculous lesion different, but we did not observe any lesions suggestive of Hodgkin's disease in the tissues. From this it became apparent that only at certain stages of acute avian tuberculosis in the rabbit did the megakaryocytes wander out of the marrow in sufficient numbers to enable their detection in numbers in the tissues.

Our next observations were on rabbits, guinea pigs and calves which were inoculated with a virulent bovine tubercle bacillus. The strain of tubercle bacillus used in this study was a culture of B.C.G. (the strain of tubercle bacillus advocated by Calmette for prophylactic vaccination against tuberculosis), which had had its virulence greatly enhanced by growing in a favorable environment *in vitro*. The rabbits and calves were inoculated intravenously and the guinea pigs subcutaneously. All of the animals in this study died in from three to four weeks from extensive acute tuberculosis.

Since the three types of animals showed essentially the same type of lesions which resembled Hodgkin's disease it is unnecessary to describe them separately. The calves showed the most marked lesions. Numerous groups of megakaryocytes were found in tuberculous lymph nodes and in hemolymph nodes. Sections of the calves' lungs often showed as high as eight to ten megakaryocytes in a single high power field. Collections of megakaryocytes in the spleen were found in each type of animal used. The liver showed a very rare megakaryocyte and on the whole was not much involved. The greatest liver involvement occurred in the guinea pig. The bone marrow was hyperplastic and showed moderate to marked hyperplasia of the megakaryocytes. The hyperplastic marrow could not be distinguished from that of the rabbit's marrow in avian tuberculosis.

Here again lesions resembling Hodgkin's disease were not observed in animals that lived a long time with a chronic tuberculosis.

Our next study was made on guinea pigs infected with virulent human tubercle bacilli. The results were the same as in our previous experiments.

Recently we have observed the same type of lesion in lung tissue of a human being who died from an extensive acute tuberculous pneumonia, having been under sanatorium treatment for over three years. The final acute spread which caused death occurred only a few days before death. Studies of the lung sections in this case showed numerous megakaryocytes in the part of the lung involved in the recent "spread" of the tuberculous process, whereas in the more chronic lesions such cells were not observed. Here again we have the presence of megakaryocytes in the acute tuberculous process and not in the chronic.

Discussion

From the illustrations which accompany this article one can see the close similarity between the megakaryocytes in the acute tuberculous animals and the Sternberg giant cells which one finds in Hodgkin's lesions in human beings. The significance of this observation will be discussed in a future article on Hodgkin's disease.

It is of interest that in acute tuberculosis caused by different types of tubercle bacilli in different animals there is a marked response on the part of the megakaryocyte as well as of the mononuclear leucocyte and the neutrophile. It is commonly observed that there is a marked increase and abnormally large platelets in the circulation of animals and of man in extensive acute tuberculosis. It is not surprising, therefore, that there is a hyperplasia and a wandering out from the bone marrow of the megakaryocytes which are the cells from which platelets are derived. There can be no question that these cells do leave the marrow and that they wander about in the tissue. They have been observed free in an alveolar space in the lung. The part that the megakaryocyte and its platelets play in the acute tuberculous process is not known. Fibrin is abundant in these acute tuberculous lesions and it may be that the megakaryocyte and the platelets play a definite rôle in fibrin formation.

The increase of platelets and the presence of megakaryocytes in acute lesions is not peculiar to acute tuberculosis. Such a condition is often seen in acute lobar pneumonia. It does not appear to be specific for any infection and in all probability it is a general biological phenomenon which occurs in acute infectious and non-infectious injury to tissue. For instance, saponin, when injected into rabbits, will cause a marked emigration of megakaryocytes from the bone marrow into the tissues, the animals dying in four or five days. The ability to produce such a condition with a drug shows clearly that the response on the part of the megakaryocyte is not dependent upon a bacterial infection.

In none of the animals which we have studied has the gross pathology suggested Hodgkin's disease. This is true whether the disease was acute or chronic. In the human case cited in this article there was no gross pathology which suggested Hodgkin's disease. To date we have been unable to find any lesions in tuberculous tissues which would suggest Hodgkin's disease, except in the histopathology of

acute tuberculosis. These lesions we believe are simply accumulations of megakaryocytes in the tissues and are not real Hodgkin's lesions. We have not observed the pleomorphism of cells, the predominance of the Hodgkin's cell type or the other phenomena which constitute the histopathology seen in a true case of Hodgkin's disease.

We do not believe that any type of tubercle bacillus is the etiological agent in Hodgkin's disease. Neither do we believe that any infectious agent is the etiological factor. The recent report by Twort [5] makes such a belief more tenable than ever before. It is possible that an acute infection may in some instances upset the normal balance in such a way that Hodgkin's disease may ensue. But it is just as plausible that such an imbalance might be produced by a non-infectious agent. As long as one adheres to the dogma that a response on the part of the hematopoietic tissues, especially of the leucocytes, designates the presence of an infection within the tissues, it will remain imperative that the leukemias, Hodgkin's disease, pernicious anemia, and so on, be ascribed to the action of some pathogenic microörganism. If the above concept is logically adhered to, then all responses on the part of the blood cells signify infection. This would lead one to believe that nature has prepared for the eventual occurrence of infections by differentiating the blood cells early in embryonic life, long before there is any contact with pathogenic microörganisms. To carry this conception one step further, all inflammatory reactions must be due to the presence in the tissues of pathogenic microörganisms. Thus an abscess formed as a result of sterile turpentine injected into the tissues is the result of pathogenic microörganisms being present and is not due to the turpentine.

If, however, one believes that the function of the blood cells is to assist in the maintenance of a biological balance compatible with life, then it becomes apparent that their rôle extends far beyond the part they take in the combat against an infectious agent. Such a biological concept offers a better explanation of the acute inflammatory reaction which is seen in an animal sensitized to a sterile protein substance such as egg white, or which is produced when a sterile chemical substance such as turpentine is injected into living tissue. Thus it can be concluded that such pathological conditions as Hodgkin's disease or the leukemias may occur without infectious

agents being the incitant as readily as if they were present. It also enables one to understand the fact that identical tissue reactions may be observed in infectious and in non-infectious injury to the living tissues.

The finding of lesions resembling Hodgkin's disease in acute tuberculosis emphasizes the fact that it is necessary to have more than giant cells of the Sternberg type to establish a diagnosis of Hodgkin's disease. One may find in an enlarged and hyperplastic lymph node such giant cells scattered about in the tissues, and yet the lesions may not be Hodgkin's disease at all.

Summary and Conclusions

1. Lesions resembling Hodgkin's disease are often present in acute tuberculous foci. These lesions are simply the accumulation of megakaryocytes which have emigrated from the bone marrow to the areas of damaged tissue. They are not true Hodgkin's lesions.

2. The participation of the megakaryocyte in an acute tuberculous process is emphasized. This is not specific for tuberculosis since it occurs in other pathological conditions.

3. The necessity of exercising care in the diagnosing of Hodgkin's disease from tissue sections is stressed. The presence of the Sternberg type of giant cell in tissue is not sufficient evidence to warrant a diagnosis of Hodgkin's disease.

4. Various types of virulent tubercle bacilli cause the production of the lesions which simulate Hodgkin's disease. This would seem to preclude the possibility that any one type of tubercle bacillus is the etiological agent of Hodgkin's disease.

5. Lesions suggestive of Hodgkin's disease have been observed only in acute tuberculosis. In chronic tuberculosis these lesions have not been encountered.

REFERENCES

1. L'Esperance, E. S. *J. Immunol.*, 1930, **18**, 127.
2. Ewing, J. Neoplastic Diseases. W. B. Saunders, Philadelphia.
3. Bunting, C. H., and Yates, J. L. *Bull. Johns Hopkins Hosp.*, 1917, **28**, 151.
4. Haythorn, S. R. Personal Communication.
5. Twort, C. C. *J. Path. & Bact.*, 1930, **33**, 539.

DESCRIPTION OF PLATES

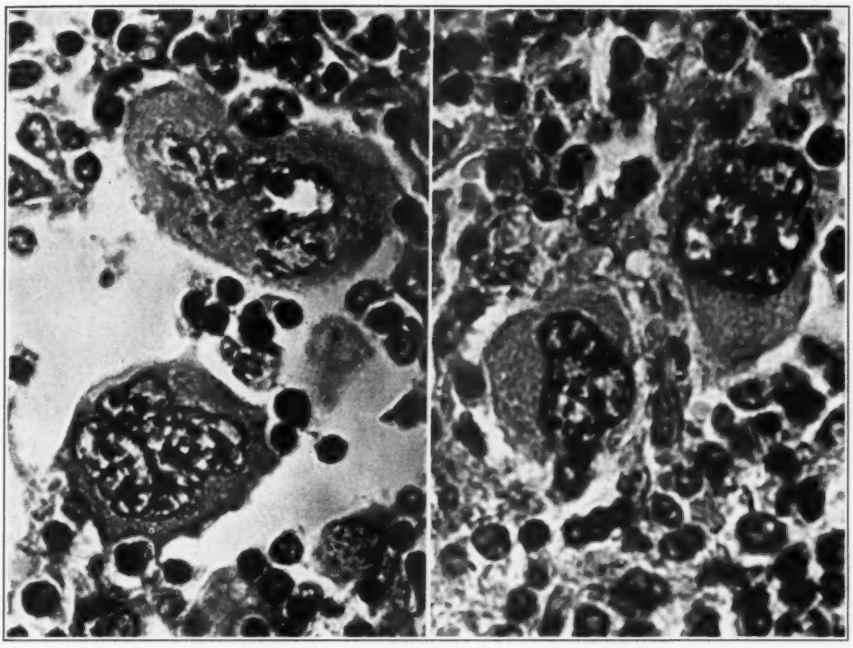
PLATE III

FIG. 1. Hodgkin's lymph node showing two Sternberg giant cells. × 1000.

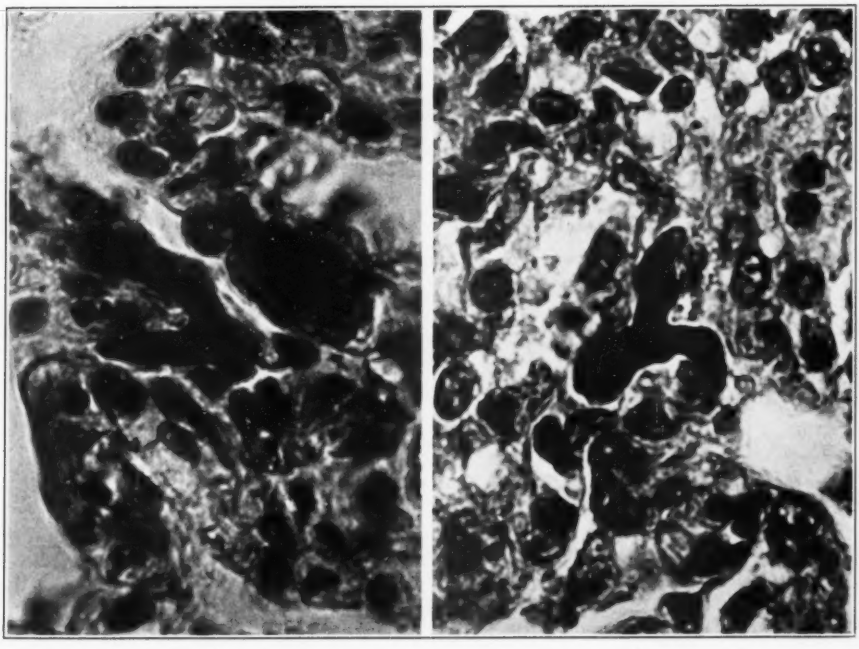
Fig. 2. Lymph node in acute tuberculosis in a calf. Two megakaryocytes in field. Bovine tubercle bacillus infection. × 1000.

FIG. 3. Lung in Hodgkin's disease without tuberculosis. Note two giant cells in alveolar walls. × 1000.

FIG. 4. Megakaryocyte in alveolar wall. Acute tuberculosis in calf. Bovine tubercle bacillus infection. × 1000.



1 2



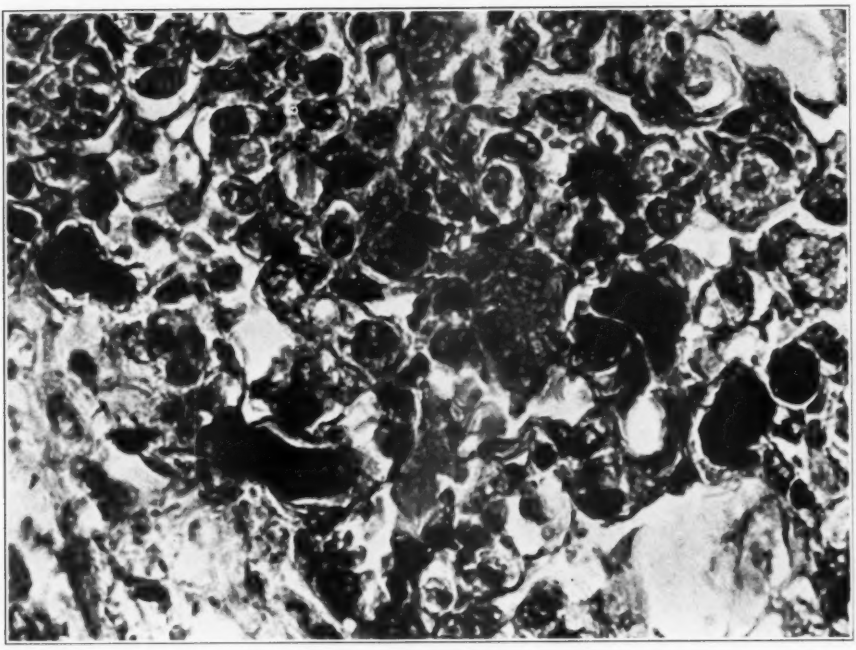
3 4

Medlar and Sasano Lesions Resembling Hodgkin's Disease

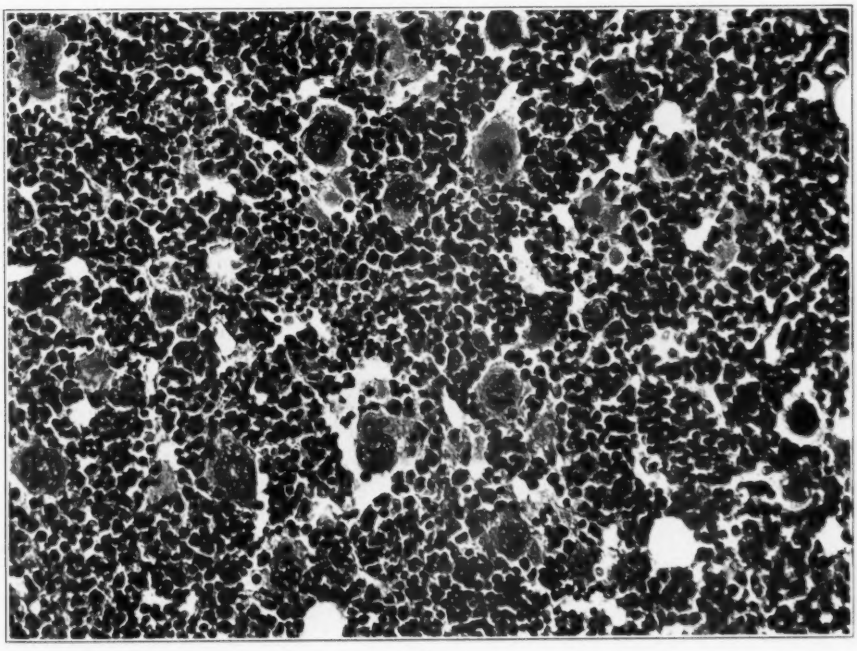
PLATE 112

FIG. 5. Lung, calf. Acute tuberculosis. Numerous megakaryocytes in field. × 1000.

FIG. 6. Bone marrow from rabbit. Marked hyperplasia of marrow. Note numerous megakaryocytes. Two megakaryocytes in mitosis to right of field. Bovine tubercle bacillus infection. × 400.



5



6

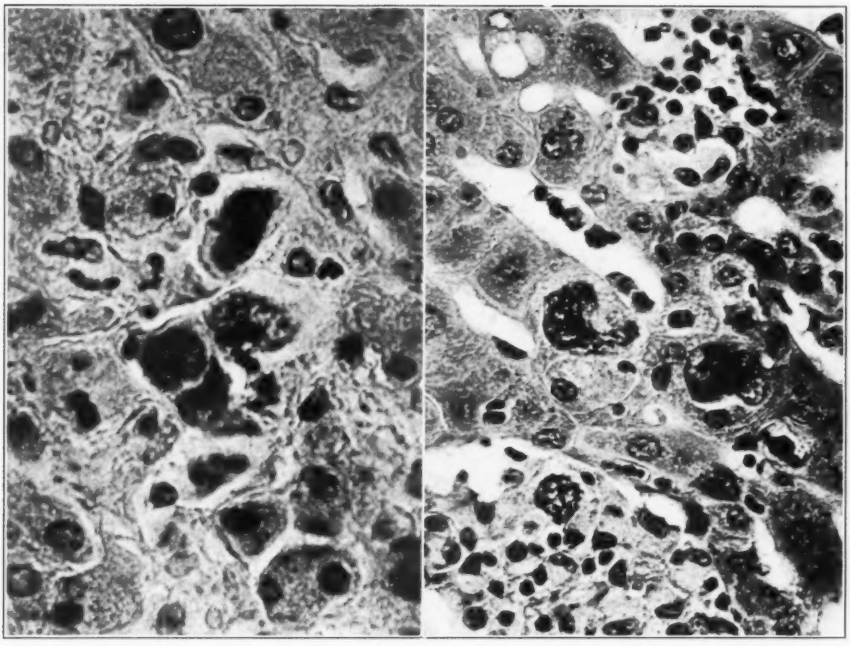
PLATE 113

FIG. 7. Human liver from case of Hodgkin's disease. Four Sternberg giant cells in center of field. × 800.

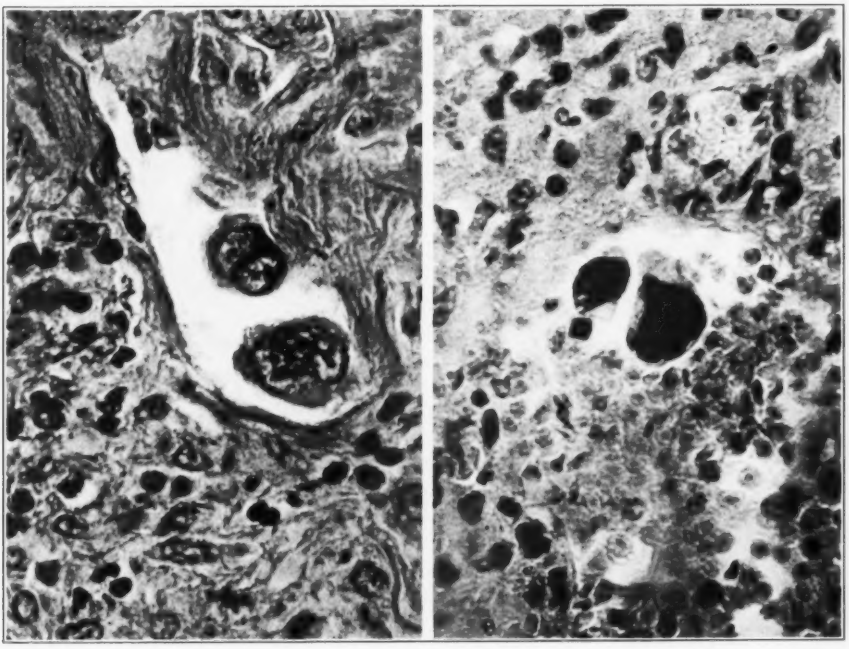
FIG. 8. Rabbit liver. Avian tubercle bacillus infection. Three megakaryocytes in field. × 800.

FIG. 9. Human spleen. Scirrhous Hodgkin's lesion. Two Sternberg giant cells in sinusoid. × 800.

FIG. 10. Guinea pig spleen. Human tubercle bacillus infection. Two megakaryocytes in large sinusoid. × 800.



7 8



9 10

Medlar and Sasano Lesions Resembling Hodgkin's Disease

Plate 114

Figs. 11, 12, 13. Rabbit marrow. Megakaryocytes in process of entering circulation. × 800.

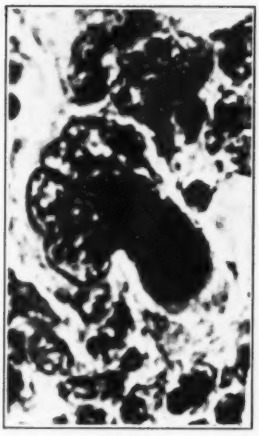
Fig. 14. Two mitotic figures in megakaryocytes in rabbit marrow. Avian tubercle bacillus infection. × 800.

Fig. 15. Megakaryocytes in mitosis. Lung from calf. Bovine tubercle bacillus infection. × 800.

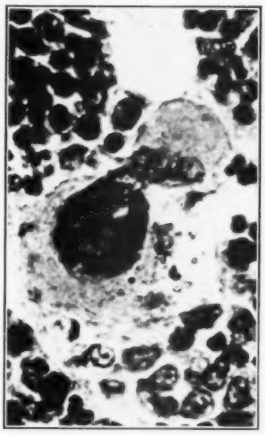
Fig. 16. Megakaryocyte in mitosis. Spleen from rabbit. Bovine tubercle bacillus infection. × 800.

Figs. 17 and 18. Megakaryocytes wandering about in tissue. Lung from calf. Bovine tubercle bacillus infection. × 1000.

Fig. 19. Megakaryocytes in alveolar wall. Acute tuberculous pneumonia in human lung. Human type tubercle bacillus infection. × 800.

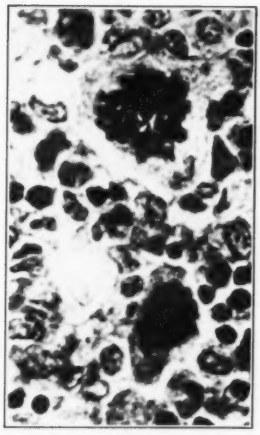


11



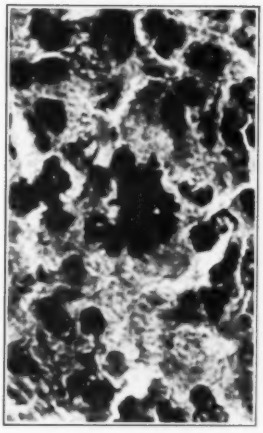
12

13



14

15



16

17

18

19

Medlar and Sasano Lesions Resembling Hodgkin's Disease

AN INTERPRETATION OF THE NATURE OF HODGKIN'S DISEASE*

E. M. MEDLAR, M.D.

(From the Hegeman Memorial Research Laboratory of the Metropolitan Life Insurance Company Sanatorium, Mount McGregor, N. Y.)

The number of contributions relative to Hodgkin's disease is so large that it is impossible to present adequately the views of the various authors in a single article, unless the paper be devoted entirely to that phase of the subject. The review of Simonds [1] renders another article on this topic superfluous at the present time, for little has been added in the way of further clarification of this disease since his article was published. Our references to the literature will, therefore, be confined to citing certain points of view pertinent to the discussion of Hodgkin's disease we desire to present.

It is generally conceded that there is a pathological and clinical entity which justifies the grouping of certain cases under the term "Hodgkin's disease." The features which allow such a separation are so well known that no comment is necessary.

The nature of Hodgkin's disease is still very uncertain. There are two schools extant at present. The one school maintains that the disease is neoplastic in nature. The adherents to this view maintain that the tumor is primary in the lymphoid tissues of the body.

The second school believes that Hodgkin's disease is of an infectious nature. Bacteriological studies by different investigators have led to no single microörganism as the etiological agent. The tubercle bacillus has been held to be the inciting agent by some authors. The reason for this deduction has been the obtaining of tubercle bacilli from Hodgkin's tissues. The irregularity of these findings has led to a belief that to obtain Hodgkin's lesions it is necessary to have a certain type of tubercle bacillus which has a certain degree of pathogenicity. More recently L'Esperance [2] has presented evidence which suggests that the avian tubercle bacillus might be the etiological factor.

* Received for publication May 30, 1931.

Other investigators, Bunting and Yates,[3] have failed to find the tubercle bacillus frequently in Hodgkin's lesions, but have found diphtheroid organisms quite consistently. More recently, Haythorn[4] has obtained a culture of monilia from the tissues of a case of Hodgkin's disease.

In the study of tuberculous lesions in experimental animals we have noted the occurrence of numerous giant cells in lungs, liver, spleen and lymph nodes which we were unable to differentiate from the giant cells seen in Hodgkin's lesions. We observed these lesions suggestive of Hodgkin's disease first in rabbits inoculated with virulent avian tubercle bacilli. Upon further study we found similar lesions in guinea pigs, rabbits and calves infected with virulent bovine tubercle bacilli. We have also observed similar lesions in guinea pig and human tissues infected with virulent human type tubercle bacilli.

A thorough study of the various tissues of the experimental animals and of human tuberculous tissue led us to the conclusion that the cells which resembled the Sternberg giant cells of Hodgkin's lesions were megakaryocytes. At certain stages of acute tuberculosis there was a marked hyperplasia of the megakaryocytes in the marrow, with a wandering-out of these cells from the marrow into the circulation and thence into the various tissues involved in the tuberculous process. We have not observed this phenomenon in chronic tuberculosis, although it is possible that it exists to a slight degree.

The marked hyperplasia of the megakaryocytes in acute tuberculosis and our inability to distinguish between the megakaryocytes in the tissues and the Sternberg giant cells in Hodgkin's lesions led us to a study of the histopathology of Hodgkin's disease, with the possibility that the megakaryocyte might be the cell type primarily involved in the pathological process.

The material which we were enabled to study consisted of twenty-two autopsies and about one hundred surgical specimens.* Unfortunately the number of bone marrow specimens which we examined were very few. Of the twenty-two autopsies, bone marrow sections were available in but six instances. In four instances femur marrow

* We wish to express our appreciation for the kind permission of Drs. F. B. Mallory and F. Parker, Jr., to utilize the autopsy material and surgical specimens of Hodgkin's disease from the pathological laboratory of the Boston City Hospital. They granted the study of the skin nodule from a case of myelogenous leukemia also.

alone had been procured and in two instances vertebral and femur marrow had been obtained at the time of autopsy.

During this study we were privileged to examine a biopsy specimen from the skin of an individual who had the clinical and blood picture of myelogenous leukemia. This individual had numerous nodules in the skin in various parts of the body. The study of the sections from this skin nodule gave the point of departure for the interpretation of Hodgkin's disease which will be presented here.

The histopathological picture present in sections of the skin nodule was complex. In places there were groups of nondescript cells (Fig. 1). These cells were round, with a round to oval nucleus and slightly granular cytoplasm. They might be called large lymphoid cells, reticular cells or simply non-differentiated cells. They probably are the parent or "stem" cell of the other cell types noted in the section. In other areas of a single section the following cells were noted in groups: neutrophilic leukocytes (Fig. 2), eosinophilic leukocytes, megakaryocytes (Fig. 3) and nucleated red blood cells (Fig. 4). In other areas these were found comingled.

From this study we considered that the lesion was the result of the differentiation of the multipotential parent cell of the marrow into the various cell types which it produced normally. In other words there were leukopoietic, erythrogenic and megakaryogenic centers all present in the skin because of the differentiation of a common parent cell into these various cell types.

Since we were interested primarily in determining the possible relationship of Hodgkin's disease to the megakaryocyte, our chief interest centered on the bone marrow. Our findings in this tissue will be given in detail since we believe that the pathology which we observed is of real significance. In brief, it may be stated that the bone marrow in every case in which we studied this tissue was pathological. We will limit our description of the histopathology to one section of vertebral bone marrow, since all of the other sections of marrow gave the same picture to a greater or lesser degree.

In our study of the marrow reaction in experimental tuberculosis in animals we traced the megakaryocyte back to a cell somewhat larger than a myelocyte. This cell has very little cytoplasm and a relatively large, deeply staining nucleus. The cytoplasm was often very granular. Since this cell appeared to be the premegakaryocyte

in animal marrow we naturally sought for the same cell type in the Hodgkin's marrow.

Fig. 5 shows the appearance of a considerable part of a section of vertebral marrow from a case of Hodgkin's disease without evidence of tuberculosis or of other infectious lesions. Note the considerable number of nondescript cells which resemble the cells shown in Fig. 1. Whether these are premegakaryocytes or "stem" cells, one cannot say with certainty. One may state, however, that here is present a hyperplastic marrow with undifferentiated cells predominant. No megakaryocytes are seen.

Fig. 6 gives a picture also commonly met with in this same section. Here are shown two groups of the immature cells and no megakaryocytes in the field.

Fig. 7 shows an area a short distance from Fig. 6. Note the three megakaryocytes of different size and different nuclear appearance. Note also the group of cells similar to those in Fig. 6 just above the smallest megakaryocyte.

Fig. 8 is a portion of bone marrow in the same part of the section as Fig. 6. Here are shown five megakaryocytes and no groups of cells as shown in Fig. 6.

Figs. 9, 10 and 11 are higher magnifications from the same general area as Figs. 6, 7 and 8, to show that in all probability the typical megakaryocyte is the result of fusion of several of the smaller cells. Note that in Fig. 10 the complicated nuclear structure is quite similar to that in Fig. 11. There is, however, no abundant and definite cell cytoplasm as in Fig. 11. Note also the distinct granular cytoplasm in all three figures. This is more clearly seen in Fig. 11.

Fig. 12 shows an area of fibrosis in the same section from which the above photomicrographs were taken. In this particular field there are no tumor cells. The tissue is made up entirely of fibroblasts and lymphocytes. This corresponds to the fibrotic areas commonly found in Hodgkin's lymph nodes.

Fig. 13 is from another part of the same section of vertebral marrow and shows the picture commonly seen in scirrhotic Hodgkin's lymph nodes. Note the ameboid shape of the Sternberg giant cell in the center.

From the above description it will be seen that all of the complex picture of Hodgkin's disease can be demonstrated in a single section of bone marrow. The points we wish to emphasize are (1) hyper-

plasia of the marrow with a marked increase of immature cells which probably are the progenitors of megakaryocytes; and (2) presumptive evidence that the giant cells (megakaryocytes) are the end-result of fusion of several premegakaryocytes.

Mitotic figures were observed quite often in the hyperplastic marrow. The majority of mitotic figures were in the small parent cell. Occasionally one found a complex multiple mitotic figure in the large megakaryocyte. These observations also hold for the lesions found in other tissues in the body.

Since the Hodgkin's lesions, as seen in the various tissues of the body, differ in no essential respect from those described above in the bone marrow, further description would seem unnecessary. There is one point relative to the tumor cells that should be emphasized, and that is their ability to wander about in the tissues. This was shown to a striking degree in some of the surgical specimens which were promptly placed in Zenker's solution or in 10 per cent formalin.

Discussion

Any deliberation on diseases in which the blood-forming tissues of the body are chiefly concerned must necessarily be many-sided because of the complexity and the distribution of the tissue under consideration. We have observed but little in the pathology of Hodgkin's disease that has not been recorded in the literature already. The interpretation of the disease which we wish to present departs in certain respects from that presented in the literature. Our interpretation is given because to us it clarifies to some degree the nature of the disease. We are well aware of the fact that this interpretation departs rather radically from precedent and from authority. However, from the data we have at hand, our deductions appear logical to us. We do not believe that our views are final and we have no intention of being dogmatic in our statements.

The histopathology found in a nodule from the skin of an individual who had the clinical and hematological syndrome of myelogenous leukemia has a very important bearing on the interpretation of Hodgkin's disease given in this article. The ability to demonstrate in a single section of this nodule the various cell types, in groups as well as comingled, which are known to be produced in the bone marrow is, we believe, of great significance. It suggests that

all of these cell types have a common parent cell. Such a view is held by many authors, Bunting,[5] Maximow[6] and others. This leads one to a belief in the unitarian theory of hematopoiesis in the bone marrow. If, as we postulate, Hodgkin's disease may be a disease in which the megakaryocyte is the cell type chiefly involved, then it becomes apparent that Hodgkin's disease is closely related, genetically, to the myeloid leukemias and to the erythroblastic dyscrasias.

If one considers the derangements of hematopoiesis in the marrow from such an angle, then one recognizes that the further the process reverts to an embryonic state the greater will be the difficulty and the uncertainty of identification of the cells seen either in sections of tissue or in blood smears. It would seem probable that the parent cell of the megakaryocyte is a cell, which in its most immature state could not be successfully differentiated from a similar cell, which may be the parent cell of the erythrocyte or of the neutrophile. In other words, the more immature the cells of the marrow, the greater the uncertainty as to what type of cell they may become in their maturation. It is commonly known that in acute leukemic conditions it is, at times, extremely difficult, if not impossible, to determine with certainty whether the disease is of the myeloid or the lymphoid type. Some cases of Hodgkin's disease have been known to develop "lymphoid" leukemia. Since, to us, it appears that Hodgkin's disease is closely related genetically to the leukemias, it is not inconsistent that the leukemic manifestation should be an integral part of the disease process. From our study it would seem plausible that in this leukemic manifestation the cells may be very immature and in such a state they might be easily confused with immature parent cells of the lymphoid tissues. Until such time as the "stem" cell of the lymphoid tissue can be unqualifiedly distinguished from the "stem" cell of the marrow, it would seem that one is not justified in being dogmatic on the subject. In such cases of Hodgkin's disease as develop a leukemic blood pressure, we suggest a careful study of the cell types to establish, if possible, the origin of the lymphoid-like cells, and not remain content with saying they are of lymphoid origin simply because we know that the lymph nodes are very commonly involved in the disease.

The report of Minot[7] is of interest relative to the presence of megakaryocytes in the circulation in cases of myelogenous leukemia. His observation of immature megakaryocytes and of abnormal plate-

lets tends further to establish the unitarian theory of hematopoiesis in the bone marrow. The finding of nucleated red blood cells may be explained on the same basis. The presence of nucleated red blood cells and of myelocytes in the blood stream in Hodgkin's disease, and of abnormal platelets and of myelocytes in cases of grave erythroblastic derangements, all point toward the probability that there is a single parent cell for hematopoiesis in the marrow. This cell must be multipotential. The great variations which are manifested in dyscrasias of the bone marrow should logically lead to a study of the factors which determine the development of the marrow cells mainly along the line of neutrophiles in one case and along the line of erythrocytes in another, rather than to a superficial and artificial separation of these manifestations into distinct pathological or clinical entities.

While a classification of the pleomorphic derangements of hematopoiesis is necessary, such a classification, as far as the bone marrow is considered, should be used merely to indicate the predominant phase of a complex pathological process in which, fundamentally, a single type, multipotential cell is concerned.

Recent reports by Furth and Stubbs [8, 9] on leukosis in fowl bear directly on our discussion. They state that they have found two distinct types of disease, myelogenous and erythroblastic. A very significant feature of their findings is that when blood from the myelogenous type is injected into normal fowl, the disease reproduced is not uniform in type. Some of the fowl develop the myelogenous type, others show the erythroblastic type, while in still other fowl injected with blood from the same source a mixed type of disease becomes manifest. The same lack of uniform results occurs when blood from the erythroblastic type is injected into normal fowl. These observations point very strongly to a common multipotential parent cell for the complex disease process which they have found in the leukosis of fowl. It is of interest, also, that they have not noted the occurrence of typical lymphocytic leukemia in their studies. This would suggest that, in the fowl at least, there is a distinct difference between the leukemias of bone marrow origin and those of lymphoid tissue origin. There is a certain amount of evidence to suggest that there is also a distinct difference between these two types in the human being. That the most embryonic types of these diseases may closely resemble each other is probable.

Symmers[10] regards Hodgkin's disease as a systemic disease in which all of the hematopoietic tissues of the body are involved. He, as well as others, has noted the marked abnormality of the bone marrow. According to this author the disease is neither infectious nor neoplastic in nature. If one establishes too rigid a formula to which all malignancies must conform, then it would seem that no dyscrasia of the blood could be considered as a malignancy. There is no tissue in the body which is so pleomorphic or so mobile as the hematopoietic tissue. Therefore, in considering this tissue it would seem inconsistent to regard it in the same way as one would the epidermis. In a malignancy of the epidermis the essential considerations are (1) the autonomy of the newgrowth, and (2) the surety of the production of epidermal cells wherever the autonomatous cells may metastasize. These criteria are present in the leukemias, erythroblastic dyscrasias and Hodgkin's disease, if one considers primarily the characteristics of the parental cell of the bone marrow. The varied cellularity of these diseases would seem to be due to the fact that the parental cell is multipotential instead of unipotential as in the case of an epithelioma.

With the above discussion in mind one may logically construct the following schematic outline of hematopoiesis.

This schematic representation includes all that is, at present, accepted facts relative to hematopoiesis and ideas which, at present, are plausible although not proved. While there are controversial points in the concept, still it gives a workable, logical formula which enables one to understand more lucidly the various manifestations one observes in abnormal hematopoietic tissues and circulating blood. A recognized controversial point, to mention but one, is the placing of the monocyte as arising from the lymphoblast. Some authors hold that this cell arises from fixed connective tissue phagocytes, while others maintain that it is of bone marrow origin. Certain observations of the author (unpublished) lead him to believe that the lymphoid tissue (including the spleen) is the seat of origin of the monocyte. In an unsolved question such as this, one must admit the possibility that any point of view may be correct until unquestionable proof is established of the exact origin of the cell. Too much reliance must not be placed, however, upon a positive oxidase reaction or a certain arrangement of supravitally staining granules, for the parental cell may not show these characteristics.

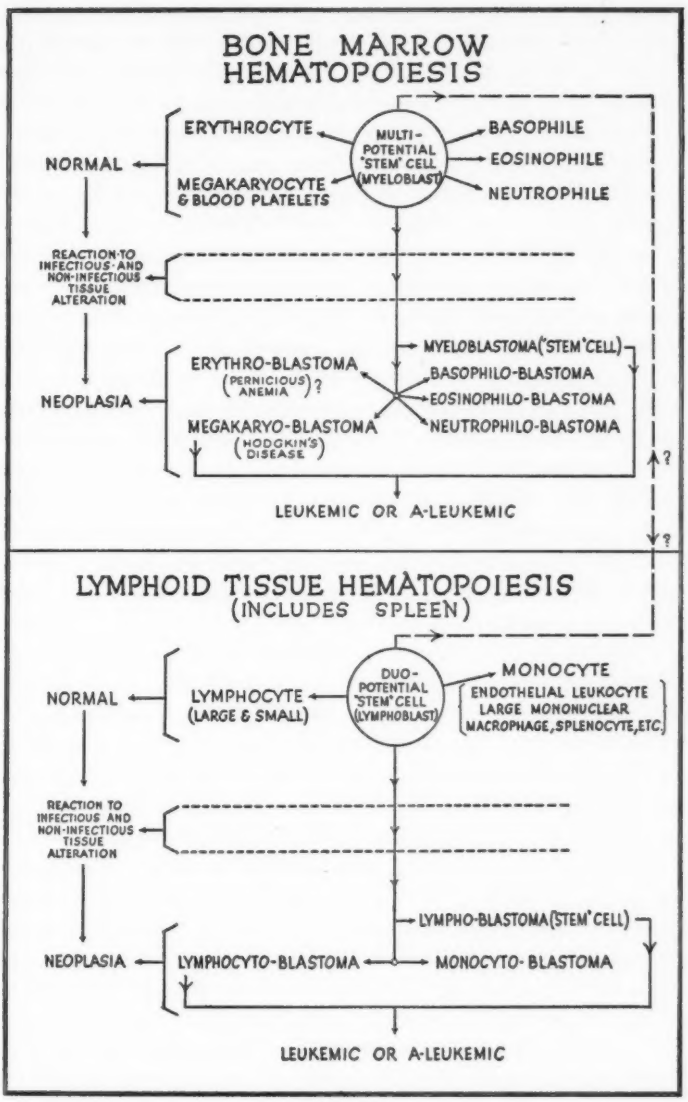

BONE MARROW
HEMATOPOIESIS
NORMAL
ERYTHROCYTE
MEGAKARYOCYTE & BLOOD PLATELETS
MULTI-POTENTIAL 'STEM' CELL (MYELOBLAST)
BASOPHILE
EOSINOPHILE
NEUTROPHILE
REACTION TO INFECTIOUS AND NON-INFECTIOUS TISSUE ALTERATION
NEOPLASIA
ERYTHRO-BLASTOMA (PERNICIOUS ANEMIA)?
MEGAKARYO-BLASTOMA (HODGKIN'S DISEASE)
MYELOBLASTOMA ('STEM' CELL)
BASOPHILO-BLASTOMA
EOSINOPHILO-BLASTOMA
NEUTROPHILO-BLASTOMA
?
LEUKEMIC OR A-LEUKEMIC
?
LYMPHOID TISSUE HEMATOPOIESIS
(INCLUDES SPLEEN)
NORMAL
LYMPHOCYTE (LARGE & SMALL)
DUO-POTENTIAL 'STEM' CELL (LYMPHOBLAST)
MONOCYTE
ENDOTHELIAL LEUKOCYTE
LARGE MONONUCLEAR MACROPHAGE, SPLENOCYTE, ETC.
REACTION TO INFECTIOUS AND NON-INFECTIOUS TISSUE ALTERATION
NEOPLASIA
LYMPHOCYTO-BLASTOMA
LYMPHO-BLASTOMA ('STEM' CELL)
MONOCYTO-BLASTOMA
LEUKEMIC OR A-LEUKEMIC

Chart 1

It will be noted that in the schematic outline under consideration a line has been drawn from the "stem" cell of the marrow to the "stem" cell of the lymphoid tissue with arrows pointing in either direction. This line is to suggest that there may be a possibility of the myeloblast assuming the function of the lymphoblast, or *vice versa*. Whether this does actually occur in mature life would appear impossible to prove since, at present, no sure differentiation can be made between a "stem" cell of the lymphoid tissue and a similar cell of the bone marrow. If it is possible for these cells to assume functions foreign to their usual rôle, then one must admit the possibility of the abnormal cells in Hodgkin's lymph nodes being derived from lymphoblasts. However, such an occurrence appears remote to the author for one does not see erythrogenic or leukopoietic centers commonly in these lesions. It would seem illogical for the lymphoblast to take over but a small part of the function of the myeloblast.

In considering such an outline as given above one can conceive that the bone marrow response in a case of severe infection might become so abnormal that a neoplastic condition would be simulated. That such conditions do arise should not lead one to hypothecate that all blood dyscrasias are the result of infection and that there is no true non-infectious neoplasia of the hematopoietic tissues. Such reasoning applied to other tissues we know would lead to erroneous conclusions. For instance, over-production of connective tissue in tissue repair we know seldom ends in a true neoplasm, although the gross and microscopic appearance might closely simulate an early neoplasm. To conclude that neoplasms never arise during the course of tissue repair we know would be without foundation. Autonomy of cell growth is a fundamental essential for neoplastic development. To recognize such autonomy at its inception is, at present, impossible. This is just as true for the hematopoietic tissue as it is for the fixed connective tissue. Different degrees of malignancy are recognized in neoplasms. This is in all probability, as true for the hematopoietic tissues as for any other tissue. The majority of medical men admit that neoplasms of the skin, bone and other tissues arise without infections playing any rôle. We can see no logical reason why the same may not be true of the hematopoietic tissue.

Those who are of the opinion that Hodgkin's disease is infectious in nature also regard all of the leukemias as being caused by some

pathogenic microörganism. We believe that such an interpretation of these dyscrasias is due to the fact that leukocytic responses of all types have come to be regarded almost wholly as indicative of an infectious process. The significance of the response on the part of the blood cells as representing a broad biological response, common to both infectious and non-infectious tissue damage, seems to have received but little acclaim. It seems that we are more easily impressed by the activities of the pathogens than by the activities of the body tissues in their attempt to adjust themselves to a wide range of changing environments. At present the neutrophile is considered pathognomonic of an acute infection by the large majority of medical men. Since the parent cell of the neutrophile appears to be the same as the parent cell of the erythrocyte, it would appear unreasonable to pick out the neutrophile any more than the erythrocyte as pathognomonic of acute infection. Yet it is doubtful if anyone would look upon the presence of immature erythroyctes in the circulation as pathognomonic of acute infection. It would seem more logical to look upon all of the cells of the hematopoietic tissue as carrying on a definite physiological or metabolic function in the body, irrespective of the presence or absence of any infectious agent. One might say that the rôle of each leukocytic type is primarily to care for damage to the body tissues of one type or another, irrespective of whether this alteration is produced by a tubercle bacillus, a pneumococcus, necrotic tumor tissues, croton oil or sterile normal salt solution. Undoubtedly the determination of the definite function of each leukocytic type far antedates the presence of any pathogenic bacterium in the tissues. Logic suggests an interpretation of the response of the marrow and lymphoid cells upon the basis of the type of alteration in the body to which they respond, rather than upon the presence or absence of infection. One grants that infectious agents damage tissue, but so will boiling water. In either instance the leukocytic response will tell with considerable accuracy the degree and type of damage the tissues have sustained. If one is willing to regard the response on the part of the hematopoietic tissue in such a light, then it becomes evident that the leukemias of one type or another may properly be removed from the infectious diseases and placed logically under neoplasms.

Many observers, Bunting,[11] and others, have noted the marked increase of blood platelets and the presence of enormous blood platelets

in the circulating blood in Hodgkin's disease. Since Wright [12] has so clearly demonstrated that megakaryocytes are the cells from which platelets arise, the findings relative to the platelets in Hodgkin's disease fit in readily with the disease being primarily an involvement of the megakaryocyte.

The finding of increased or abnormal platelets is not peculiar to Hodgkin's disease. Such a phenomenon is also noted in acute lobar pneumonias, in acute tuberculosis and other acute processes. It would appear that blood platelets play a far more important rôle in the response to acute damage to tissues than is generally appreciated.

The finding of neutrophilic or eosinophilic infiltration in Hodgkin's lesions need not necessarily indicate the presence of an infectious agent. Their presence can just as logically be explained by the necrosis of the tumor cells.

Another common occurrence in Hodgkin's lesions is fibrosis. This occurs in bone marrow, as well as in lymph nodes and other tissues. From a purely theoretical consideration one may explain this fibrosis in the following way. If the tumor cell type is the megakaryocyte, then there will be a marked production of blood platelets in the tumor nodules. Blood platelets are closely connected with the formation of fibrin. Wherever there is much fibrin formation there is an organization of the fibrin by fibroblastic proliferation. This would lead to the marked fibrosis commonly seen in the more chronic Hodgkin's lesions.

By placing the primary lesion of Hodgkin's disease in the bone marrow one can more easily understand the irregular distribution of the lesions in the body. Lesions outside of the marrow may be considered metastatic tumor growths. These metastases, in all probability, take place through the blood stream. From a study of the circulating blood which we are pursuing at the present time, we have a certain amount of evidence that this is true. This study will be reported at a later date. Minot [7] has also noted immature megakaryocytes in the blood in Hodgkin's disease. Regarding the lymph node involvement as a metastasis through the circulation, one can understand the independent and spontaneous enlargement of lymph nodes, simultaneously or at different dates, in different parts of the body such as the groin and the axilla. Why the tumor finds lymphoid tissue especially suitable for metastatic growth remains at present an unsolved question. The same is true of the reason why

some lymph nodes or other tissues, such as the liver, are involved more frequently than others.

A study of the lymph node lesions gives evidence that the consecutive enlargement of lymph nodes in a certain area, such as the cervical nodes, is due to metastasis from one lymph node to its neighbor. One can quite frequently find numerous groups of the tumor cells within lymph ducts.

In lymph nodes, especially in early growth, one finds the tumor cells scattered in different parts of the organ with the architectural structure of the node fairly normal. Older lesions will show practically the whole of the node occupied by the tumor growth. If it is appreciated that the megakaryocyte can wander about in the tissues, then it is easy to understand the diffusion of the tumor throughout the node in the early stages.

Since megakaryocytes are commonly found in tissues in diseases other than Hodgkin's disease, diagnosis cannot be made by simply finding giant cells of the Sternberg type. Rather it is essential to have a pleomorphism of cells which represent the developmental cycle of the megakaryocyte. This pleomorphism ranges from the small parent cell to the typical megakaryocyte.

One finds in hyperplastic marrow in disease other than Hodgkin's the pleomorphism of cells representative of the developmental cycle of the megakaryocyte. This phenomenon, however, is much more prominent in the bone marrow of Hodgkin's disease than in other hyperplastic marrows. We have not found in tissues outside of the bone marrow the pleomorphism of cells representative of the developmental cycle of the megakaryocyte in diseases other than Hodgkin's disease or in certain cases of "stem" cell leukemia of the bone marrow. While neutrophilic and eosinophilic infiltration and fibrosis are commonly met with in Hodgkin's lesions, they need not be present. Their presence is not necessary to establish the diagnosis of Hodgkin's disease from a section of tissue.

The study of the developmental cycle of the megakaryocyte in the marrow in acute tuberculosis has led us to appreciate that megakaryocytes do not necessarily arise as such. One finds the small immature cell, which apparently is the forerunner of the typical megakaryocyte, and the next thing one observes readily in the hyperplastic marrow is the typical megakaryocyte. From a study of the marrow in Hodgkin's disease a certain amount of pre-

sumptive evidence is found which suggests that the small premegakaryocytes fuse to form the typical megakaryocyte. Such a finding allows an understanding of the difficulty in demonstrating various stages between the premegakaryocyte and the typical adult cell. It would seem logical to consider the premegakaryocyte or the immature megakaryocyte as analogous to the thrombocyte in the blood of birds and reptiles. Minot regards megakaryocytes with small nuclei as degenerative forms. From our observations the presence of several small nuclei in a megakaryocyte is not a criterion of degeneration of the cell.

From the above discussion it would seem as though Hodgkin's disease should be removed from the group of infectious diseases and placed as a malignancy of the hematopoietic tissues of the bone marrow. This also would imply the removal of this disease as a primary neoplasm of the lymphoid tissue. The involvement of tissues outside of the bone marrow could be logically regarded as metastatic growths.

A certain amount of evidence is presented which suggests that the cell primarily involved in the pathology of Hodgkin's disease is the megakaryocyte. To denote that the predominant phase of Hodgkin's disease, which distinguishes it from the other bone marrow dyscrasias, is the primary involvement of the megakaryocyte, the term "megakaryoblastoma" is suggested to designate real Hodgkin's disease.

Summary and Conclusions

1. Evidence is presented which suggests that Hodgkin's disease is a malignancy of the bone marrow. The type cell appears to be the megakaryocyte.

2. The developmental cycle of the megakaryocyte is presented. It would seem that the typical megakaryocyte is the result of fusion of several premegakaryocytes.

3. The histopathology of Hodgkin's disease is a pleomorphic aggregation of cells which represent the developmental cycle of the megakaryocyte. It is not essential to have fibrosis or eosinophilic or neutrophilic infiltration to establish the diagnosis of Hodgkin's disease.

4. The involvement of lymph nodes and other tissue outside of the bone marrow appears to be metastic tumor growth.

5. Evidence is presented which tends to prove that all blood cells arising from the marrow have a common parent cell.

6. The term "megakaryoblastoma" is suggested to designate true Hodgkin's disease.

REFERENCES

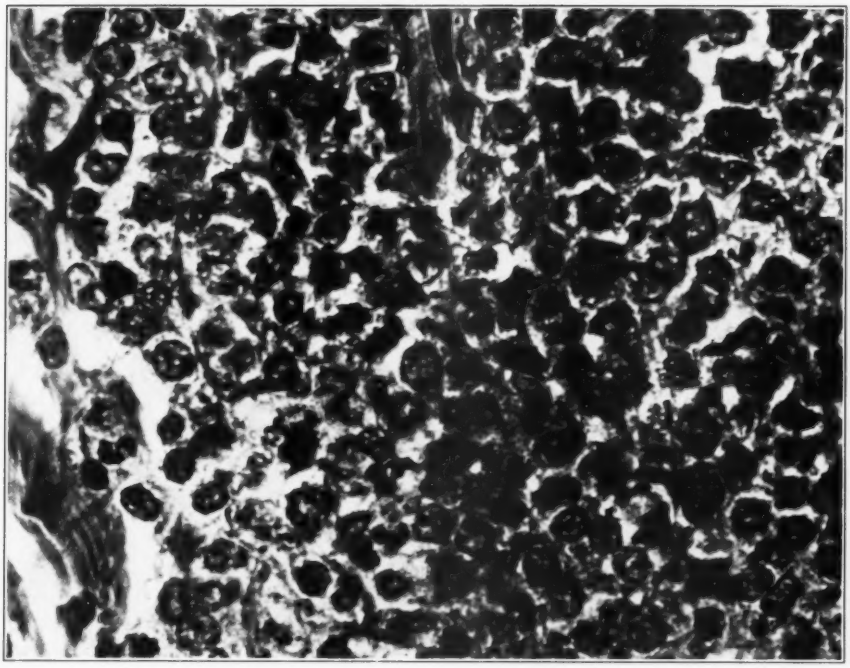
1. Simonds, J. P. *Arch. Path & Lab. Med.*, 1926, **1**, 394.
2. L'Esperance, E. S. *J. Immunol.*, 1930, **18**, 127.
3. Bunting, C. H., and Yates, J. L. *Arch. Int. Med.*, 1913, **12**, 236.
4. Haythorn, S. R. Personal Communication.
5. Bunting, C. H. *Physiol. Rev.*, 1922, **2**, 505.
6. Maximow, A. A. *Physiol. Rev.*, 1924, **4**, 533.
7. Minot, G. R. *J. Exper. Med.*, 1922, **36**, 1.
8. Furth, J. *J. Exper. Med.*, 1931, **53**, 243.
9. Stubbs, E. L., and Furth, J. *J. Exper. Med.*, 1931, **53**, 269.
10. Symmers, D. *Arch. Int. Med.*, 1917, **19**, 990.
11. Bunting, C. H. *Bull. Johns Hopkins Hosp.*, 1911, **22**, 114.
12. Wright, J. H. *J. Morphol.*, 1910, **21**, 263.

DESCRIPTION OF PLATES

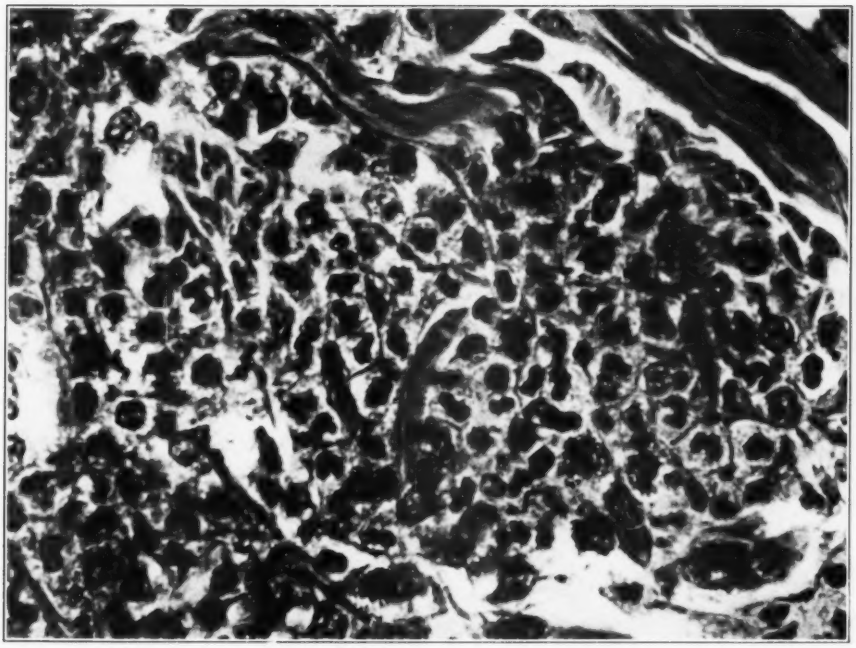
PLATE 115

FIG. 1. Area of undifferentiated cells in a skin nodule from a case of myelogenous leukemia. × 800.

FIG. 2. Area of neutrophiles in same lesion as Fig. 1. Note a few eosinophiles and a megakaryocyte in lower part of field. × 800.



1



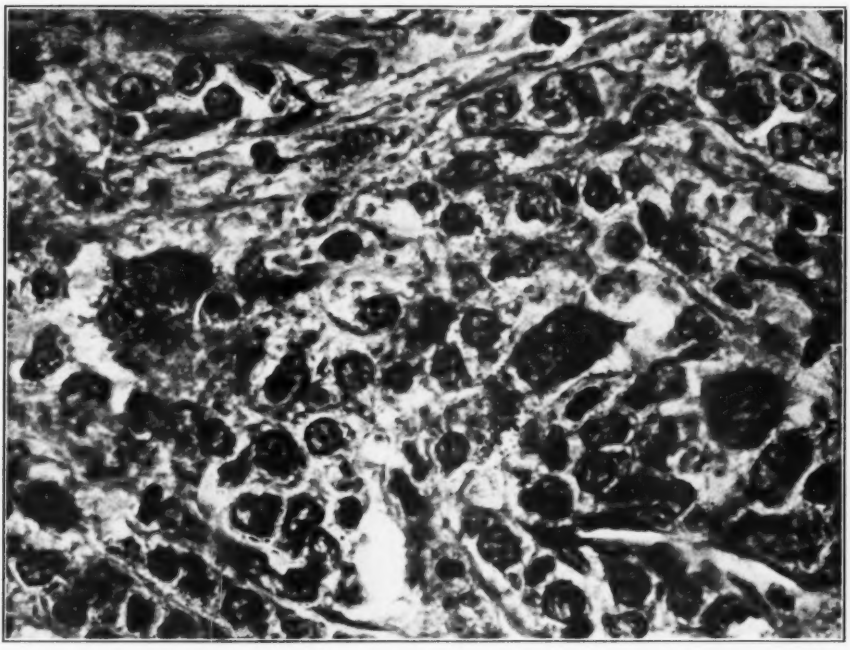
2

Medlar Nature of Hodgkin's Disease

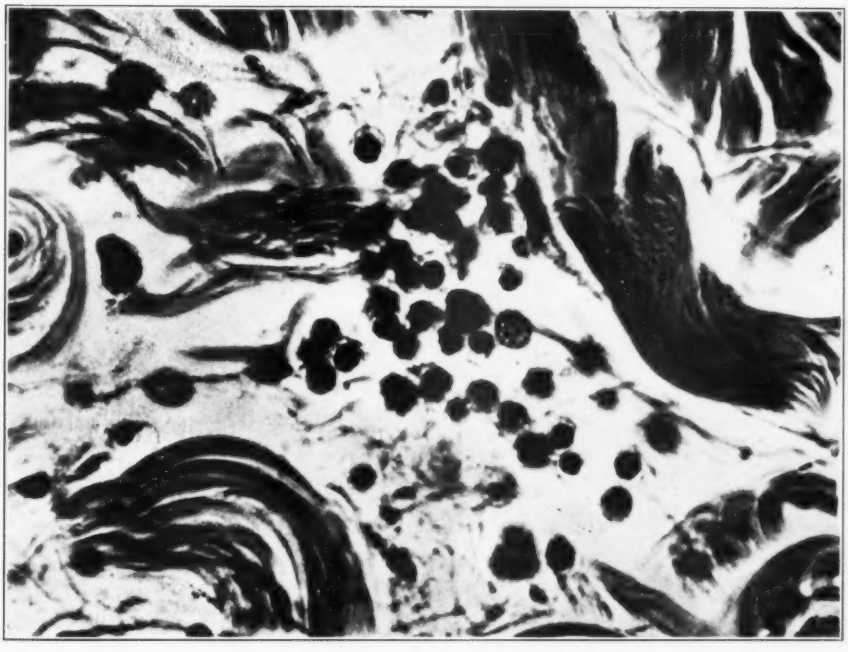
PLATE 116

FIG. 3. Megakaryocytes in same lesion as Fig. 1. Note eosinophile just below second megakaryocyte from right. Note also the numerous projections at the periphery of the megakaryocytes. × 800.

FIG. 4. Erythrogenic center in same lesion as Fig. 1. Note the nucleated red blood cells and the undifferentiated parent cells. × 800.



3



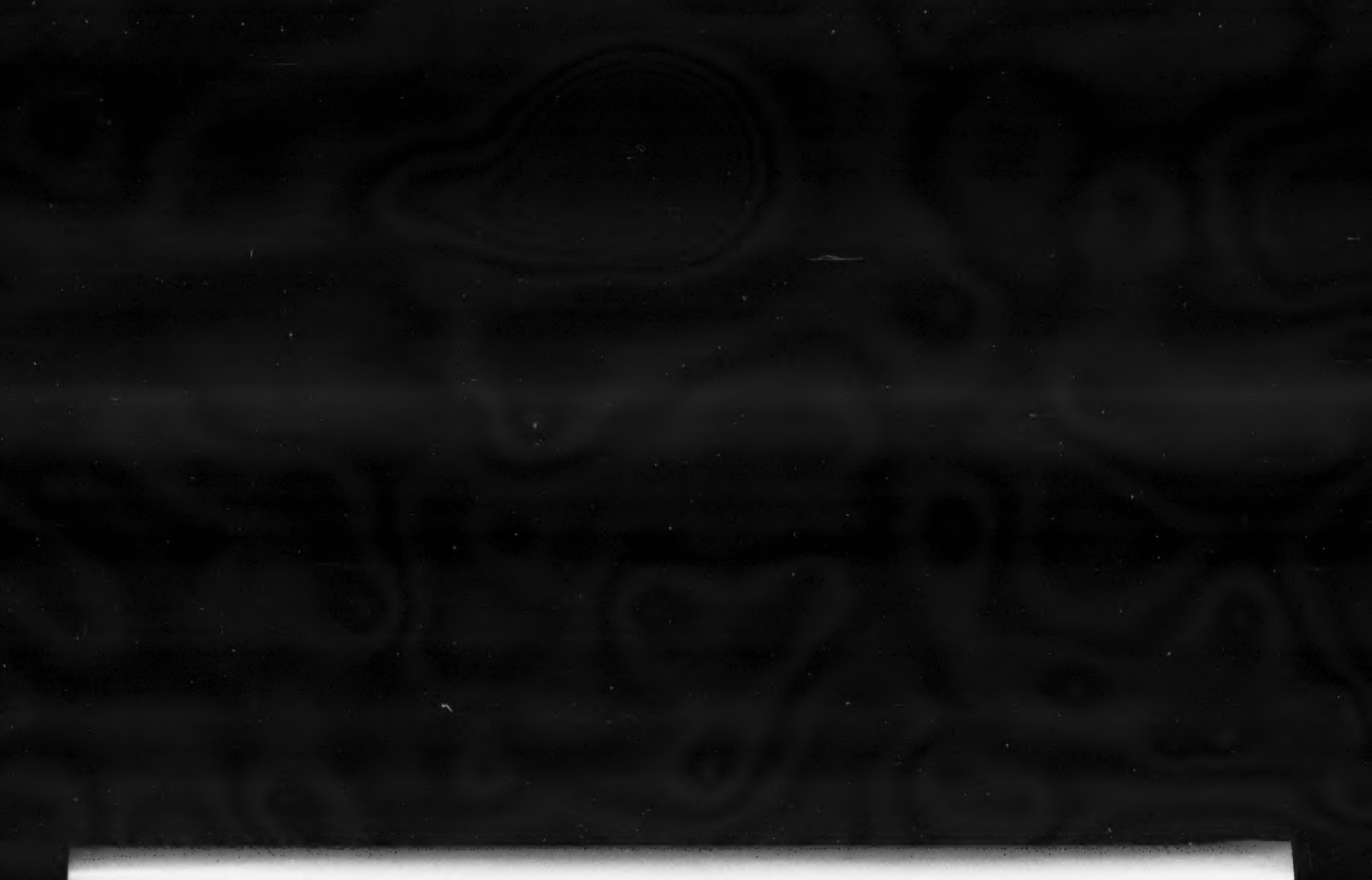
4

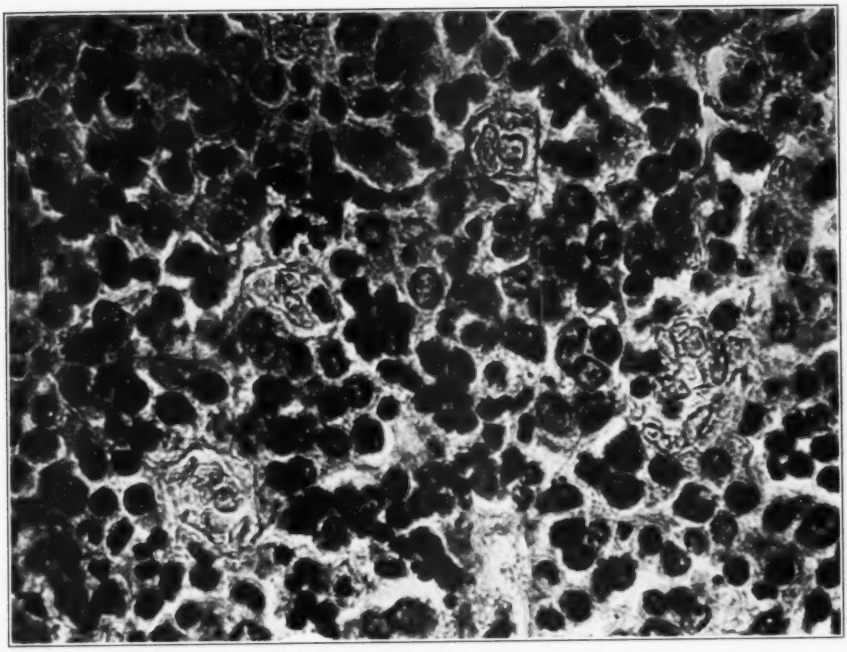
PLATE 117

FIGS. 5 to 13 are all from a single section of vertebral marrow from a case of Hodgkin's disease.

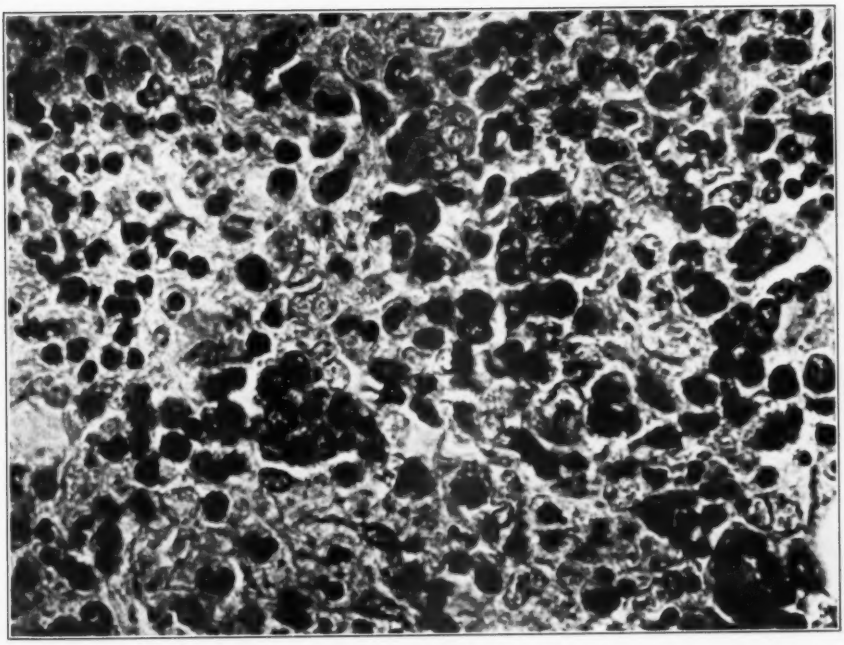
FIG. 5. Hyperplastic marrow showing undifferentiated cells, quite similar to those shown in Fig. 1, scattered throughout the field. No megakaryocytes present. × 800.

FIG. 6. Note two distinct, and one indistinct clump of undifferentiated cells in central portion of field. No megakaryocytes. × 800.





5

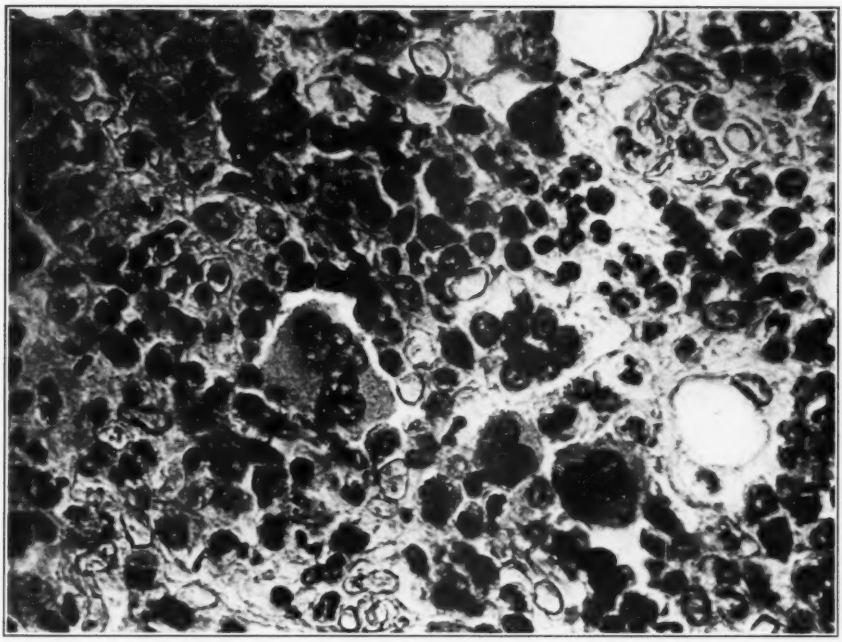


6

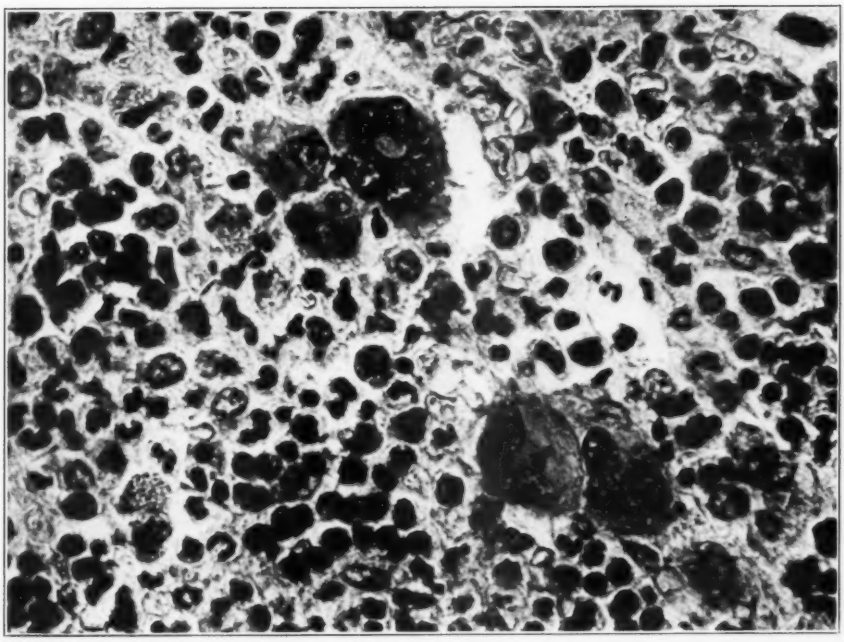
PLATE 118

FIG. 7. Three megakaryocytes and three clumps of cells in field. × 800.

FIG. 8. Six megakaryocytes in field. No clumps of cells. × 800.



7

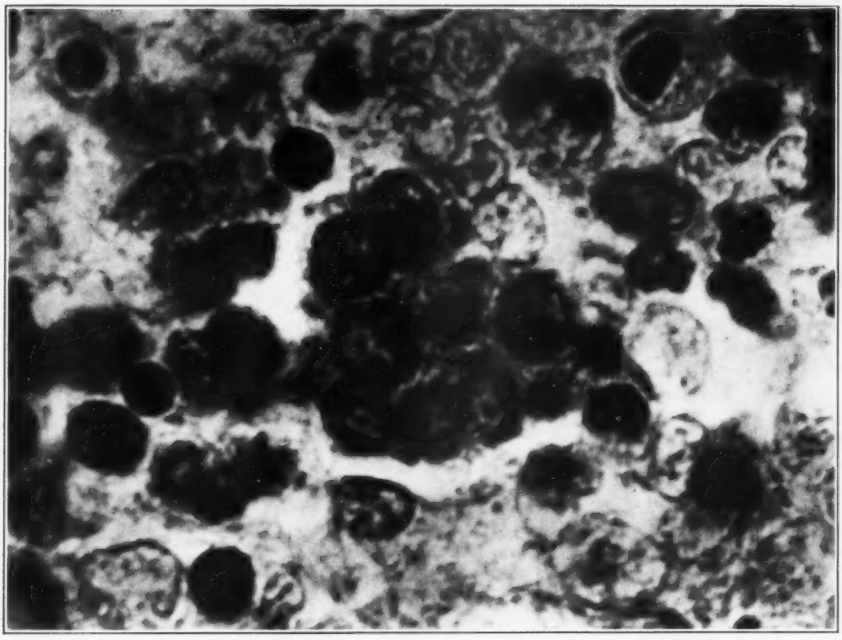


8

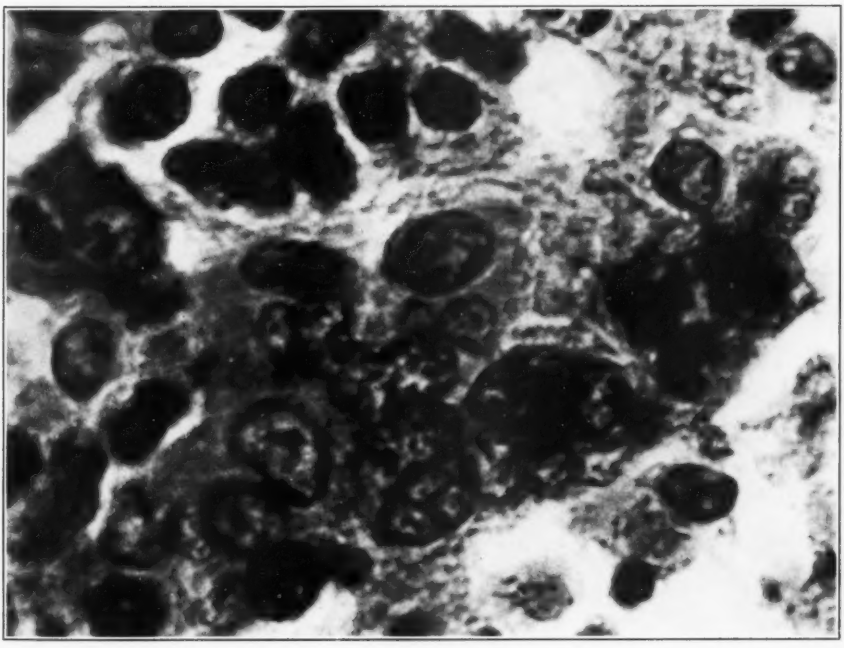
PLATE 119

FIG. 9. Clump of cells in Fig. 6. Note granular cytoplasm and separate nuclei. × 1500.

FIG. 10. Note apparent fusion of nuclei giving nuclear structure quite similar to that in Fig. 11. Note granular cytoplasm. Note also that cytoplasm is much less than in Fig. 11. × 1500.



9

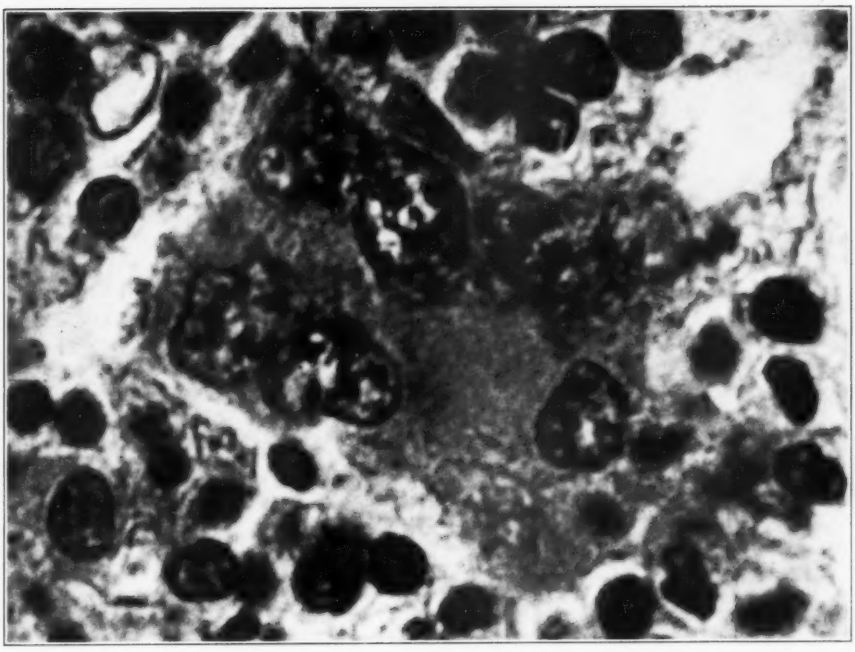


10

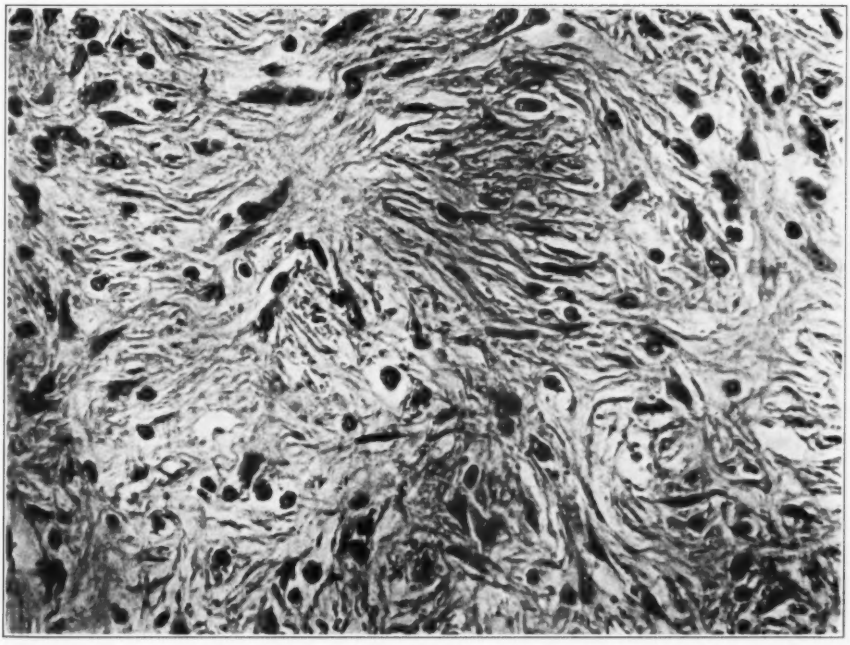
PLATE 120

FIG. 11. Megakaryocyte; nuclei are not all fused. Note abundant and granular cytoplasm. × 1500.

FIG. 12. Area of fibrosis; tissue composed entirely of fibroblasts and lymphocytes. × 500.



11



12

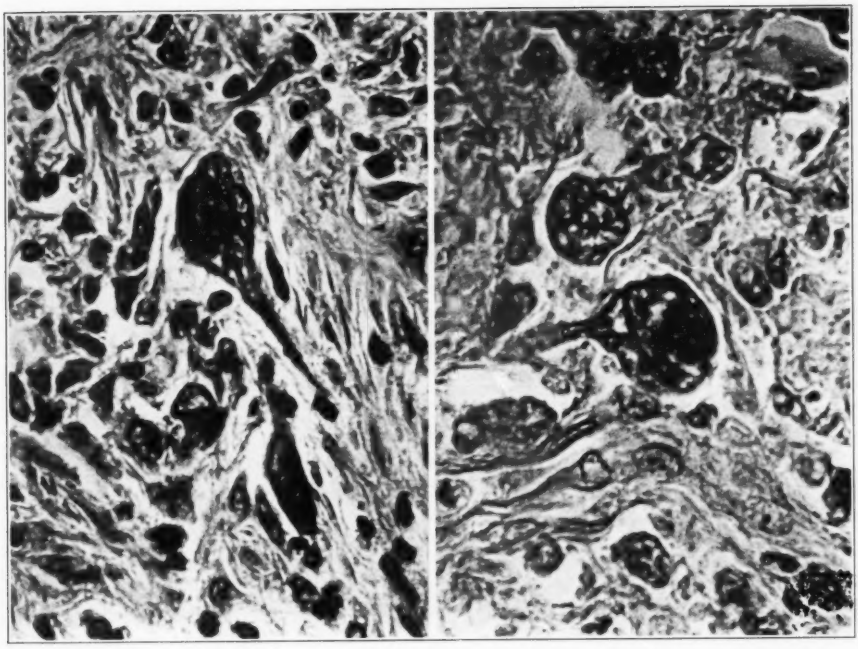
PLATE 121

FIG. 13. Typical scirrhous Hodgkin's lesion from same section as above photomicrographs. Note ameboid shape of giant cell in field. × 800.

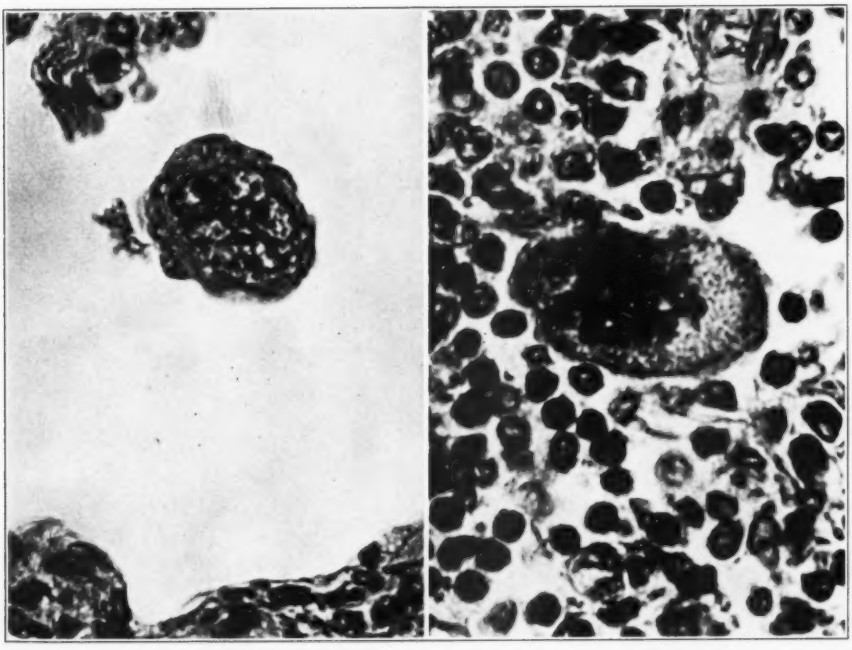
FIG. 14. Scirrhous Hodgkin's lesion in lymph nodes. Giant cells with pseudopod to left. × 800.

FIG. 15. Sternberg giant cell free within alveolar space of lung. × 800.

FIG. 16. Complex mitotic figure in Sternberg giant cell in lymph node. × 1000.



13 14



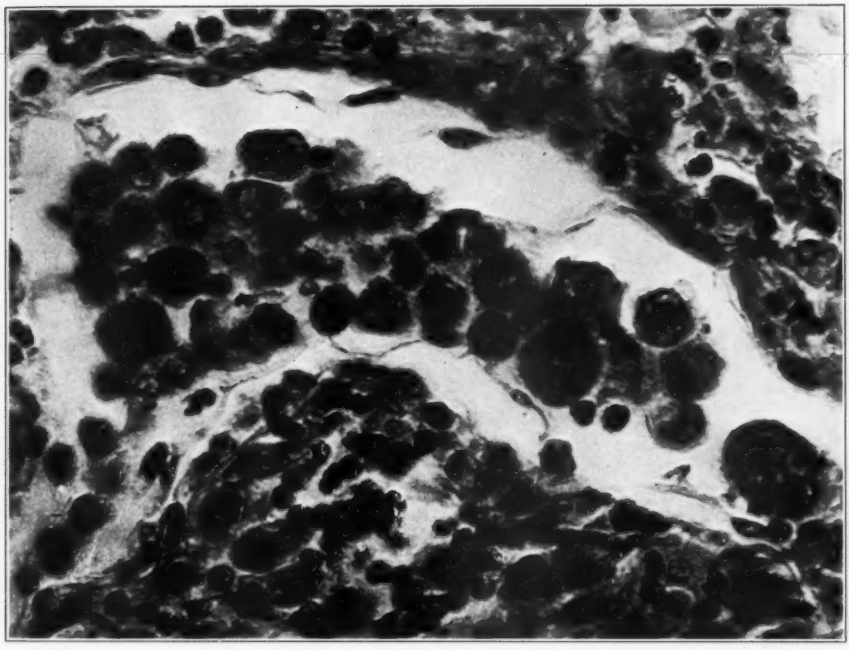
15 16

Medlar Nature of Hodgkin's Disease

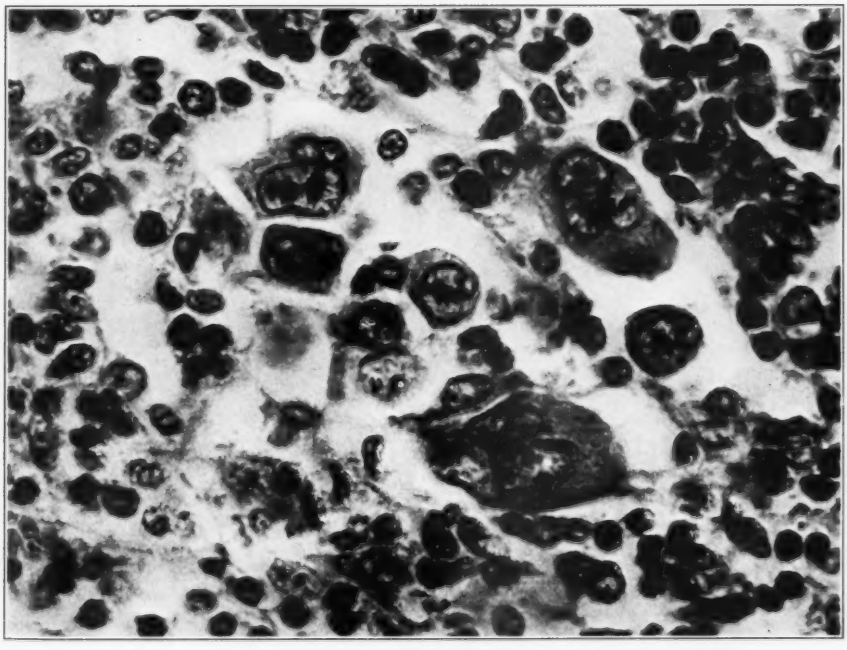
PLATE 122

FIG. 17. Large lymph sinus in lymph node filled with Hodgkin's tumor cells. Note pleomorphism of cells. × 1000.

FIG. 18. Small area from same section as Fig. 17 showing a predominance of Sternberg giant cells. × 1000.



17

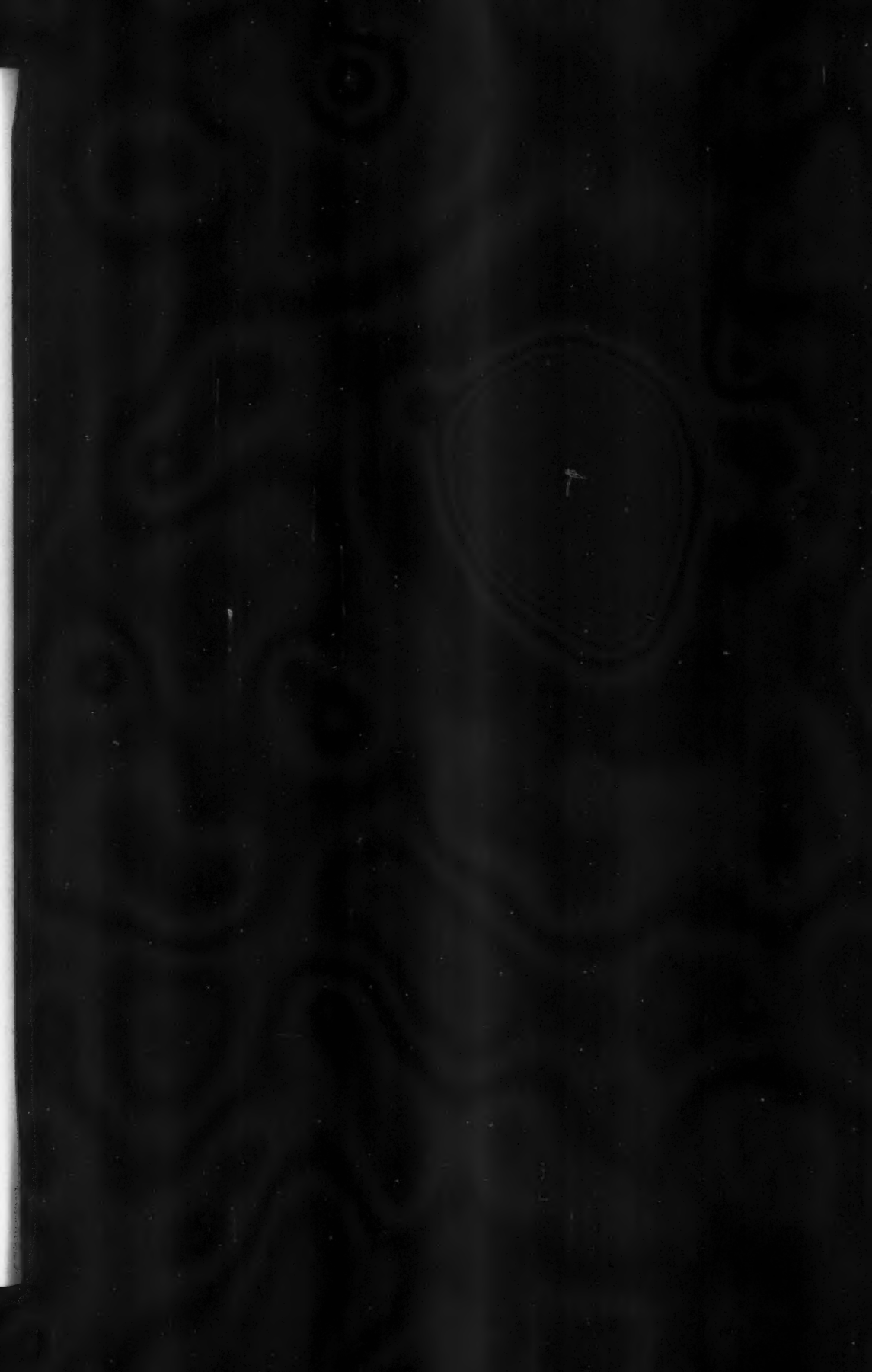


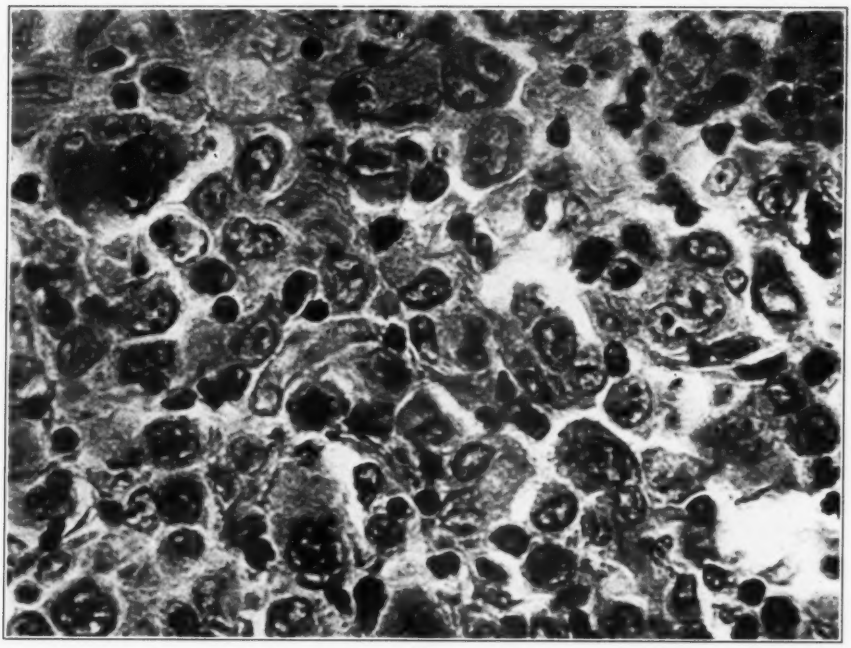
18

PLATE 123

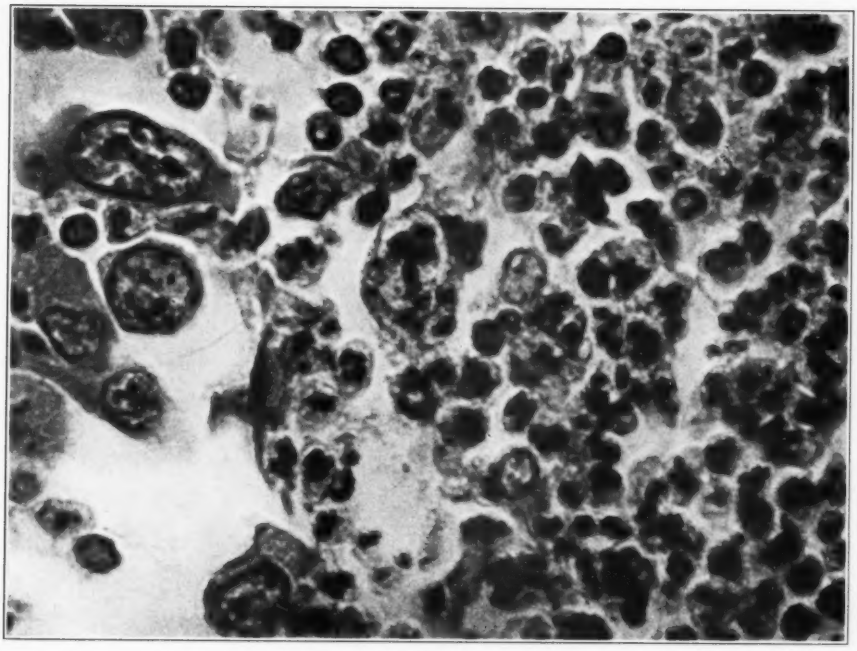
FIG. 19. Typical morphology of tumor cells in Hodgkin's disease. These cells represent the developmental cycle of the megakaryocyte. Mitosis just above center of field. Such a histopathological picture we regard as typical and as sufficient to justify a diagnosis of Hodgkin's disease. × 1000.

FIG. 20. Another area from same section as Fig. 19. Shows an area of necrotic tumor heavily infiltrated with neutrophiles. × 1000.





19



20

AN INVESTIGATION OF SPIROCHETOSIS OF THE DENTAL ANLAGE IN CONGENITAL SYPHILIS *

THOMAS J. HILL, D.D.S.
(From the Institute of Pathology, Western Reserve University, Cleveland, Ohio)

The dental defects that are found clinically associated with congenital syphilis were demonstrated by Jonathan Hutchinson [1] and Edouard Fournier [2] many years ago. Since that time considerable controversy has been centered around both the significance and the cause of these defects.

Much evidence has accumulated to support the clinical observation that a definite deformity is associated with congenital syphilis. Principally due to the contributions of Cavallaro,[3] Stein [4] and Karnosh,[5] the morphology of these defects has been well established. Some investigators have attributed the cause of this characteristic malformation to a spirochetosis of the tooth germ. Pasini [6] in 1905 and Köhler [7] in 1913 claimed to have been able to demonstrate the *Treponema pallidum* in developing enamel organs. Cavallaro [8] in 1909 published an extensive work entitled, "Syphilis and its Relation to Dentition." The English translation of this work by Schaffner states "The *Spirocheta pallida* is abundantly found in the dental follicles of developing teeth," whereas the original article states, "*La Spirochaeta pallida* s'e trovata constantemente assente, sia nel follicolo dentario, sia nella polpa dentaria, sia nel periostio alveolodentale di soggette eredo-sifilitici." This we interpret as, "The *Spirocheta pallida* are constantly absent in dental follicle, the dental pulp and alveolo-dental periosteum in subjects with congenital syphilis." In 1925 Cavallaro [9] published "Le Sindromi Sifilitiche Boccali" in which he states that his earlier conclusions were based upon the staining technique of Hoffman, but later by the use of the May-Grünwald-Giemsa stain he had been able to find the organisms, as had Pasini at a previous date. Cavallaro's article of 1925 includes a drawing of the dental pulp with spiral organisms within the blood vessel wall.

* Received for publication April 18, 1931.

Köhler [7] claimed to have been able to demonstrate the organism by the use of a silver impregnation after formalin fixation and decalcification in 5 per cent nitric acid. The staining of the *Treponema pallidum* requires a difficult and exacting technique, and decalcification in 5 per cent nitric acid renders the technique very uncertain. Römer [10] has observed a degeneration of the enamel organ in congenital syphilitics which he thought probably was due to the direct invasion of spirochetes, but makes no reference to the finding of the organism. Josefson [11] believed the disturbance in calcification was due to an interference in the function of the glands of internal secretion and attributed it specifically to the thyroid gland. Because of questionable technical procedures employed by Köhler, and insufficient evidence presented by Cavallaro, uncertainty and doubt have constantly existed.

In an attempt to settle this uncertainty, nine suspected congenital syphilitic full-term fetuses * have been examined. The enamel organs of the central incisor and the first molar of the permanent dentition were dissected from the mandible under a dissecting microscope. These tissues, with a specimen of liver from the same fetus, were fixed in neutral 4 per cent formaldehyde. Slides were prepared according to the methods of Levaditi, Warthin-Starry, Jahnel and Giemsa. The liver and enamel organs were treated at the same time, and sections of each were mounted on the same slide. Every effort has been made to give the enamel organs and the liver identical treatment.

Spirochetes morphologically similar to *Treponema pallidum* were demonstrated in the liver of six fetuses. One hundred and fifty slides from the six proved congenital syphilitic fetuses were examined and, with the exception of one structure whose identity as a spirochete is questionable, the *Treponema pallidum* was not found within the dental tissues.

Although the staining of *Treponema pallidum* in tissues is, as a rule, difficult, the fact that they have been demonstrated in the livers of these fetuses renders it extremely unlikely that the absence of the organisms in the tooth germ can be attributed to technical difficulties. It is possible that the spirochete in the tooth germ is as

* The author thanks Dr. David Seecof and Dr. Paul Gross for aid in securing material.

difficult to stain as those in acquired syphilis and that six cases are insufficient to exclude their presence.

Because the *Treponema pallidum* is more commonly found in and about the wall of blood vessels, one would expect that occasional organisms might be found in the dental pulp, but not within the avascular enamel organ. Hypoplasia of the enamel can not be explained as the result of spirochetosis unless the organisms can be found intimately associated with the areas of degeneration which have been demonstrated within the developing enamel organ in congenital syphilitic fetuses.

The results of this investigation indicate that there is insufficient evidence to justify the conclusion that the characteristic congenital syphilitic dental deformities are the direct result of the invasion of the enamel organ by the *Treponema pallidum*.

I wish to express appreciation to Dr. Howard T. Karsner for his aid and advice in this work.

REFERENCES

1. Hutchinson, J. On the influence of hereditary syphilis of the teeth. *Medical Times* (London), 1859, **1**, 697.
2. Fournier, Edouard. Des stigmates dystrophiques de la syphilis héréditaire. Thése de Paris, 1897–98, 391.
3. Cavallaro, G. La Sifilide in rapporto alla Dentizione. G. Ramella & Co., Firenze, 1908.
4. Stein, J. Dental stigmata of hereditary syphilis. *Dental Cosmos*, 1913, **55**, 695.
5. Karnosh, L. J. Histopathology of syphilitic hypoplasia of the teeth. *Arch. Dermat. & Syph.*, 1926, **13**, 25.
6. Pasini, A. *Cf.* Le Sindromi Syphilitiche Boccali. Pantello and Petrelli, Spoleto, 1925, 38.
7. Köhler, L. Histologische Untersuchungen am kongenital-luetischen Zahnkeim. *Deutsche Monatschr. f. Zahnh.*, 1913, **31**, 47.
8. Cavallaro, J. Syphilis in its relation to dentition. *Dental Cosmos*, 1908, **50**, 1161, 1325; 1909, **51**, 16, 156.
9. Cavallaro, G. Le Sindromi Syphilitiche Boccali. Pantello and Petrelli, Spoleto, 1925.
10. Römer, O. Die Pathologie der Zähne. Handbuch der speziellen pathologischen Anatomie und Histologie, Henke, F., and Lubarsch, O, 1928, **4**, pt. 2, 176. Julius Springer, Berlin.
11. Josefson. Die Pathologie der Zähne. Handbuch der speziellen pathologischen Anatomie und Histologie, Henke, F., and Lubarsch, O., 1928, **4**, pt. 2, 397.

INFLAMMATORY ADENOMATOID HYPERPLASIA OF THE MAJOR DUODENAL PAPILLA IN MAN *

V. J. Dardinski, M.D.

(From the Section on Pathologic Anatomy, The Mayo Clinic, Rochester, Minn.)

It is generally admitted that inflammatory adenomatoid hyperplasia is of widespread occurrence in the human body. In such locations as the mucous membrane of the gastro-intestinal tract, uterus and nares it produces diffuse overgrowth, or single or multiple polypoid excresences which constitute definite clinical entities, and are a feature of the late stages of chronic catarrhal inflammation of these tissues. The process affects both glands and stroma. The glands become hypertrophic or cystic and the lining cells are increased in size and number. The stroma shows new and tortuous blood and lymph vessels, multiplication of stroma cells and infiltration by lymphocytes. The process tends first to produce a diffuse thickening, but focal overgrowth of the tissue, edema, venous stasis and mechanical traction lead to papillary and polypoid outgrowths which may become numerous and bulky. In this condition there is a tendency to further overgrowth of the predominating element, glands, stroma or vessels. In the nares the mucous polyps show wide variations in structure, some being glandular, others myxomatous, and still others angiomatous, but the tendency to true tumor growth is slight.

In a recent anatomical study of the major duodenal papilla in man it was noticed that in a series of forty-eight specimens obtained at autopsy, twenty-three contained what appeared to be a polypoid structure in the outlet (Figs. 1 and 2).

Such a high frequency of occurrence aroused further interest and justified further study, especially when the literature failed to reveal any evidence that this condition has received due recognition. Letulle and Nattan-Larrier,[1] however, mention the occasional projection of the ends of the longitudinal mucous folds of the ampulla through the outlet.

* Received for publication May 1, 1931.

Using a larger series of cases, the following results were obtained. Of 100 specimens obtained at autopsy fifty-four contained a fold or folds of mucous membrane, polypoid in nature, protruding through the orifice of the duodenal papilla and apparently partially obstructing the opening. In size and shape these polyp-like structures varied from one with a single knob to one which protruded 3 mm. beyond the orifice and which was divided into several parts; each part was definitely knob-like at the extremity. In thirty-two of these fifty-four specimens there was definite congestion around the orifice of the papilla, and occasionally in the projecting mass, suggesting a definite inflammatory process.

In every specimen in which congestion of the orifice was found there was also swelling of the wall of the papilla in this region (Fig. 1). The congestion, however, was never found to extend more than 3 or 4 mm. along the intestinal surface of the papilla.

On opening these specimens along the longitudinal axis, avoiding cutting the growth at the orifice, it was found that the folds in the ampullary portion of the common bile duct were prominent and that where congestion was present they were covered with thick mucus. Also, the polypoid character of the masses in the orifices was emphasized by enlargement or swelling of the tips of these folds (Figs. 2 and 3).

In the specimens in which the common bile duct and the pancreatic duct each had a separate opening at the outlet of the papilla, these swollen folds at the orifice were confined to the common bile duct and such change was not present in the pancreatic duct. In those cases in which the ducts united 1 to 2 mm. or more from the orifice of the papilla these swollen folds were always on the side of the common bile duct. Microscopic study of these folds, using hematoxylin and eosin, Van Gieson's connective tissue stain and the mucicarmine stain, demonstrated these structures to be composed of dilated acini filled with thick mucus (Fig. 4). Each fold contained several of these acini varying in size. Their epithelial lining was composed of tall, columnar cells on a thin basement membrane of connective tissue. The cytoplasm of the cells contained mucus confined in the free end of the cell. The nuclei, as a rule, were confined to the bases of the cells and were fairly large, lightly staining vesicular structures. Other acini contained a small amount of thin mucus and short columnar cells with a small amount of cyto-

plasm, which sometimes contained mucus. The nuclei were small and heavily stained. No two acini appeared to have the same shape, the same amount of mucus, or to be composed of cells of similar size.

As has been mentioned, in the specimens in which definite congestion was present there was also enlargement of the wall of the papilla at the outlet. On section, this thickened wall was found to contain dilated acini similar to those in the swollen ends of the folds, and collections of lymphocytes on the surface and in the connective tissue.

The presence of these swollen folds in the orifice of the ampulla was suspected of producing some obstruction to the outflow of bile, and, therefore, should produce some relative dilatation of the common bile duct due to increased pressure within the duct. Therefore, the circumference of the common bile duct was measured at the level of the union of the right and left hepatic ducts, halfway between this level and the intestine, and immediately outside of the intestine.

The average circumference of the common bile duct at the juncture of the right and left hepatic ducts midway between this level and the duodenal wall, and immediately outside of the duodenal wall in the specimens with the supposed obstruction in the outlet of the papilla, were 1.6, 1.4 and 1.2 cm. respectively; while in the cases without hypertrophy and obstruction at the outlet, the measurements in the same regions were 1.5, 1.3 and 1.1 cm. respectively.

From these observations it is apparent that there is no appreciable effect on the size of the common bile duct, such as would be manifested by definite obstruction.

This might be explained by the fact that if there is any increase in intraductal pressure it is taken up by the gall-bladder.

What effect this condition has, if any, on the outflow of bile could not be determined on the dead subject, but it might possibly explain the retarded or delayed bile flow which is often encountered in the living subject.

Correlation of the changes that have been described with other changes in the body was not constant, neither was there suggestive correlation with any other associated factors. In the fifty-four cases the following more important conditions were present: hypertrophy of the prostate gland in nineteen cases; adenoma of the thyroid gland in six; polyps in the stomach, colon or uterus in thirteen; carcinoma of the colon, rectum or stomach in twelve; and leiomyoma of the uterus in four. Of these, gallstones confined to the gall-

bladder were present in five, and ulcer in the stomach or duodenum in eleven.

The most outstanding fact in the abnormality of the papilla is the sex incidence. In the fifty-four cases in which this condition was present thirteen patients were women and forty-one men. This bears the same proportional relation to sex as carcinoma in this region.

The age at which this change was found ranged from 36 to 96 years. Most of the patients, however, were in the fourth, fifth and sixth decades.

Summary

1. In 54 per cent of 100 cases in which autopsy was performed, definite changes were found in the major duodenal papilla. The changes apparently were benign and did not cause definite symptoms.

2. Enlargement of the ends of the folds and thickening of the papillary wall were due to the presence of hyperplastic mucous glands, resulting probably from repeated attacks of inflammation at the outlet of the papilla.

REFERENCE

1. Letulle, M., and Nattan-Larrier, L. Région vatérienne du duodénum et ampoule de Vater. *Bull. et mém. Soc. anat. de Paris*, 1898, **12**, 491–504.

DESCRIPTION OF PLATE

Plate 124

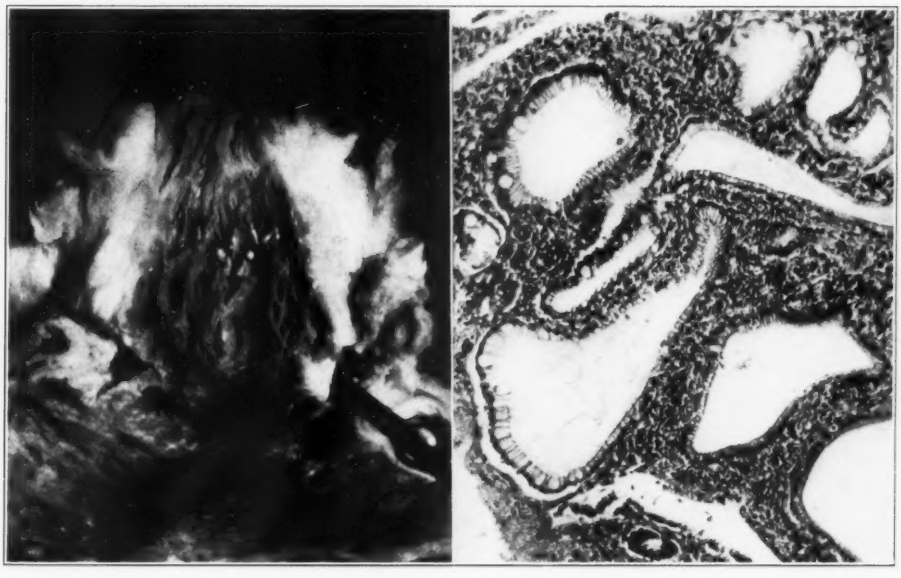
Fig. 1. The major duodenal papilla. There is a polypoid structure in the orifice, and thickening of the wall. The orifice does not appear to be occluded in this particular specimen.

Fig. 2. Outlet of the major duodenal papilla. The swollen end of one fold containing numerous mucus-secreting acini is seen. The framework of this swelling is dense connective tissue. There is definite thickening of the wall of the papilla on both sides. This is also due to dilated glandular acini, as can be seen in the picture: "*a*" common bile duct, "*b*" pancreatic duct.

Fig. 3. Interior of the major duodenal papilla. The longitudinally coursing folds of the mucosa are seen.

Fig. 4. Higher power of Fig. 3 through the swollen end of one of the folds in the outlet of the papilla to show the greatly dilated acini. Hematoxylin and eosin stain. × 150.

1 2



3 4

Dardinski Adenomatoid Hyperplasia of Duodenal Papilla

THE EFFECT OF TESTICLE EXTRACT ON THE ROUS SARCOMA *

DONALD C. HOFFMAN, M.D., FREDERIC PARKER, JR., M.D., AND THOMAS T. WALKER, M.D.

(From the Pathological Laboratory, Boston City Hospital, Boston, Mass.)

In recent publications, Duran-Reynals [1, 2, 3] has shown that there exists in the testes of normal rabbits a substance, extractable in water or saline, which has the remarkable power of greatly enhancing skin lesions due to vaccine virus and to staphylococcus. This finding was confirmed by one of us [4] working with vaccine virus and extending the work to determine the effect of this substance on the viruses of herpes, vesicular stomatitis and Borna disease. In each case the resultant infection was greatly enhanced. McClean,[5] working with vaccine virus, reported similar results. Pijoan [6] was able to enhance the lesions produced by twenty different bacteria by means of this substance. Ledingham and Barratt [7] have referred to this enhancing agent as the "Reynals' factor." Hoffman and Duran-Reynals [8] reported the fact that this extract failed to enhance the lesions produced by certain toxins and enzymes.

All the references thus far are concerned solely with the effect of testicle extract upon infections, toxins and enzymes. As to the effect of this substance on cell activity, there have been references to the stimulation of cell growth *in vitro* by testicle as well as other organ and tissue extracts.[9, 10, 11] The effect of testicle extract on cell behavior *in vivo* is referred to in papers by Aievoli,[12] and Rho,[13] who reported a stimulation of healing in chronic ulcers by means of such a substance.

On the other hand, Duran-Reynals [14] recently reported that the Brown-Pearce rabbit tumor was greatly inhibited by incubating the tumor cell suspension with testicle extract at 37° C for from two to three hours prior to inoculation. This is the only reference that can be found dealing with the effect of testicle extract on tumor growth.

It was felt, therefore, that it would be of considerable interest to carry out an experiment to determine what effect, if any, this enhancing substance would have upon the Rous sarcoma (Chicken

* Received for publication July 18, 1931.

Tumor I). Since this tumor is practically the only one to grow from cell-free filtrates, and since there has been considerable question as to whether this is a true tumor in the sense of mammalian tumors or whether it is due to an infectious agent or enzyme, it was thought that such an experiment might shed some light on this much disputed subject.

Experiment I

Methods and Materials

The tumor used in these experiments was obtained through the courtesy of Dr. H. B. Andervont of the Harvard Medical School. The tumor supplied us was finely minced, taken up in a small amount of Locke's solution and injected intramuscularly into a young Plymouth Rock hen. After ten days the tumor had grown to a large size. The hen was killed and the tumor removed sterilely. It was then finely minced with scissors, passed through a food chopper and ground in a sterile mortar with sand. One portion of this tumor mass was diluted to a 1:5 suspension with Locke's solution and was called "tumor mash." The remainder was diluted 1:10 with Locke's solution, centrifuged, and the supernatant fluid passed through a Berkefeld N filter. This material was called "tumor filtrate."

Testicle extract was freshly prepared from testes of normal, healthy, adult rabbits. The testes were removed aseptically,* the fat was dissected off, and they were finely minced and ground with sterile sand. Sufficient Locke's solution was added to make a 1:10 suspension. This material was centrifuged at high speed for fifteen minutes and the supernatant fluid was passed through a Berkefeld N filter. This filtrate was tested for sterility before use.

Twelve Plymouth Rock chickens, 8 weeks old, were used in the experiment, each chicken receiving four intradermal inoculations in the skin of the breast. Before inoculation, mixtures consisting of equal parts of tumor mash and saline, tumor mash and testicle extract, tumor filtrate and saline, and tumor filtrate and testicle extract were prepared sterilely. The scheme of inoculation was as follows: upper left area received 0.5 cc. of the tumor mash-saline emulsion; upper right area received 0.5 cc. of the tumor mash-testis extract emulsion; lower left area received 0.5 cc. of the tumor

* All operations on animals were carried out under full ether anesthesia.

filtrate-saline suspension; and lower right area received 0.5 cc. of the tumor filtrate-testis extract suspension. Each inoculated area was marked off by painting a square about 2.5 by 2.5 cm. to 3 by 3 cm. around the site of injection with an alcoholic solution of gentian violet.

It was noted at the time of the inoculations that those mixtures containing testicle extract spread through the skin extremely rapidly, whereas those wheals containing only saline persisted for a considerable length of time.

Results

Four days after inoculation of the materials the chickens were examined for the first time. At that time, in each case, small tumor nodules had begun to form in the lower left and lower right regions, where tumor mash had been injected with testicle extract, and its control containing the same amount of mash plus saline. In each case except one, the tumor nodule resulting from the injection of mash plus testicle extract was from one-and-a-half to ten times as large as the corresponding control lesion. The areas which had received tumor filtrate had not shown tumor growth at that time. The tumors were measured every two days thereafter, and each time the areas which had received testicle extract in the inoculum showed much larger tumors than the controls. Those which had received tumor filtrate showed tumors for the first time ten days after inoculation. In each case with these tumors, the regions which had received testicle extract showed multiple small nodules which covered a considerably larger area than the corresponding controls. The fourteenth day after inoculation was the last day on which all chickens were still alive. The individual measurements and descriptions of the various tumors are recorded in tabular form in Table I.

It can be seen from the table that in each case the tumor resulting from the injection of mash plus testicle extract was larger than the corresponding control—in many cases markedly so. These tumors were rapidly growing and formed one nodule which was easy to measure in its two diameters. The approximate plane area of each nodule was determined and the average calculated for all the chickens. Based on average areas, the tumors resulting from mash plus testicle extract were 3.37 times as large as those arising from mash

plus saline. This is, of course, only an approximate figure and, if anything, shows less than the real enhancement phenomenon.

TABLE I

Description of Tumor Nodules 14 Days After Inoculation

Chicken No.	Tumor mash 0.25 cc. plus Saline 0.25 cc.	Tumor filtrate 0.25 cc. plus Testicle ext. 0.25 cc.	Tumor filtrate 0.25 cc. plus Saline 0.25 cc.	Tumor filtrate 0.25 cc. plus Testicle ext. 0.25 cc.
	cm.	*cm.*		
1	1.5 x 1.7	2.3 x 2.0	3 small nodules	multiple nodules
2	3.0 x 1.5	6.0 x 4.0	few small nodules	multiple nodules filling marked area
3	3.5 x 2.0	5.0 x 4.0	few superficial and discrete nodules	same as No. 2 but extending subcutaneously
4	1.5 x 1.3	2.5 x 2.0	few small discrete nodules	several small nodules
5	1.5 x 1.0	1.5 x 1.2	1 small subcutaneous nodule	1 small subcutaneous nodule
6	2.0 x 1.0	2.5 x 2.0	3 small nodules, largest 0.4 cm. diam.	several small nodules, largest 0.5 cm. diam.
7	3.5 x 2.0	5.0 x 4.0	few discrete nodules	multiple nodules filling marked area
8	2.0 x 1.7	5.0 x 4.0	few localized nodules	multiple small nodules
9	1.5 x 1.3	2.0 x 2.0	1 small nodule	multiple small nodules filling marked area
10	3.5 x 1.8	5.5 x 3.5	few localized nodules	multiple small nodules
11	2.0 x 1.5	3.5 x 4.0	1–2 small nodules	several nodules
12	1.5 x 1.2	2.5 x 2.0	2 small nodules	multiple nodules
Average area in square cm.	3.5	11.8	..	..

On the other hand, the tumors resulting from injection of tumor filtrate consisted in almost every case of several small nodules, either discrete or confluent. A fair measurement of these was not possible. Suffice it to say that those nodules occurring following injection of

tumor filtrate plus testicle extract, showed approximately the same degree of enhancement as the ones growing from tumor mash. The striking feature was the development of a multiplicity of small confluent nodules in the extract areas which was never observed in the case of the nodules arising from tumor filtrate plus saline.

After the death of the chickens, autopsies were performed on each and bits of the skin nodules were fixed in alcohol-formalin. Celloidin sections were made from each nodule and stained with hematoxylin and eosin. Each section showed typical Rous sarcoma, but no differences histologically could be made out in the various tumors. Most of the chickens showed metastatic nodules in lung and liver.

Thus it was seen that there had occurred marked enhancement in the growth of these tumors when rabbit testicle extract had been used as a diluent for the tumor mash or filtrate.

However, there seemed to be a possibility that this enhancement might have resulted either wholly or in part from the presence of foreign protein (rabbit protein) rather than the testicle extract principle itself. Also, the question arose as to whether extract of rooster testis would exercise the same effect as rabbit testis. Consequently, a second experiment was designed to clarify these points.

Experiment II

Methods and Materials

The chicken tumor used in the second experiment was likewise procured fresh from Dr. Andervont at the Harvard Medical School and inoculated into the breast muscle of a young Plymouth Rock hen. After ten days, the hen was killed and the tumor removed aseptically. The procedure for preparing the tumor injection material was the same as in the first experiment with the exception that no tumor mash was used and the filtrate dilution was 1: 5.

The same rabbit testicle extract was used as in Experiment I.

Rooster testicle extract was made in exactly the same manner as rabbit testicle extract.

Normal rabbit serum was obtained by bleeding a normal rabbit from the heart and collecting the blood in a sterile tube. After it had clotted and the clot separated, the tube was centrifuged at high speed for twenty minutes and the serum was pipetted off.

As in the first experiment the injection masses were prepared before inoculation as follows: mixtures of equal parts of tumor filtrate and Locke's solution were made in one tube, equal parts of tumor filtrate and rooster testicle extract in another, equal parts of tumor filtrate and rabbit serum in another, and equal parts of tumor filtrate and rabbit testicle extract in the last.

Ten healthy young Plymouth Rock roosters, 8 weeks old, were used. Each received four intradermal injections according to the following scheme: upper left area, 0.5 cc. of the filtrate-Locke's solution mixture; lower left area, 0.5 cc. of the filtrate-rooster testicle extract mixture; upper right area, 0.5 cc. of the filtrate-rabbit serum mixture; and lower right area, 0.5 cc. of the filtrate-rabbit testicle extract mixture.

At the time of the inoculations it was observed that the mixture which contained rabbit testicle extract spread very rapidly through the tissues, whereas the other three mixtures left a wheal for an appreciable length of time.

Results

The first observations were made eight days after inoculation. By this time every chicken had developed tumors in each area. In each instance but one, the tumors growing after inoculation of filtrate plus rabbit testicle extract were considerably larger than the Locke's solution control. In the other areas no such constant difference in the size of the tumor nodules was apparent. In practically every case the control area was the smallest.

The tumors were measured at two-day intervals and the thirteenth day after injection was the last day when all animals were still alive. The tumors were growing rapidly, and this day was selected for recording the measurements in tabular form. These appear in Table II.

As in the first experiment, the tumors resulting from the injection of tumor filtrate plus rabbit testicle extract were in each case larger than the Locke's solution control tumors. In addition they were also larger than the rooster testicle extract and rabbit serum tumors. In want of a better method of getting an average size for comparison, the plane areas were again computed and averaged. As can be seen from the table the average area of the tumors resulting from injection of tumor filtrate plus rabbit testicle extract was 12.67 square

cm., whereas the Locke's solution control tumors averaged 3.9 square cm. The rooster testicle extract tumors averaged 3.2 square

TABLE II

Description of Tumor Nodules 13 Days After Inoculation

Chicken No.	Tumor filtrate 0.25 cc. plus Locke's sol. 0.25 cc.	Tumor filtrate 0.25 cc. plus Rooster testicle ext. 0.25 cc.	Tumor filtrate 0.25 cc. plus Rabbit serum 0.25 cc.	Tumor filtrate 0.25 cc. plus Rabbit testicle ext. 0.25 cc.
	cm.	*cm.*	*cm.*	*cm.*
13	2.5 x 2.0	3.0 x 2.0	2.6 x 3.0	5.0 x 3.5
14	1.5 x 1.3	0.9 x 1.0	1.7 x 2.1	deep extension 5.0 x 5.0
15	3.7 x 2.0	3.0 x 1.4	2.4 x 1.9	deep extension 6.4 x 2.3
16	2.8 x 2.0	2.8 x 1.8	2.7 x 2.5	4.0 x 2.7
17	0.4 x 0.4	1.4 x 0.4	2.2 x 2.2	deep and diffuse 5.5 x 2.8
18	3.2 x 1.5	2.3 x 1.4	3.0 x 3.0	deep extension 3.5 x 4.3
19	0.5 x 0.5	0	1.0 x 1.0 0.2 x 0.2	3.0 x 1.7
20	1.5 x 1.3	2.5 x 1.2	7 confluent nodules, largest 0.7 x 0.6	large confluent nodules, deep extension 2.5 x 1.8
21	2.5 x 2.1	2.8 x 2.0	2.8 x 2.8	ulcerated 4.0 x 3.2
22	2.0 x 1.9	2.5 x 1.4	deep extension 2.3 x 1.7	deep extension 4.0 x 2.5
Average area in square cm.	3.9	3.2	5.05	12.67

cm., and the rabbit serum tumors averaged 5.05 square cm. It would seem, therefore, that the mere presence of rabbit protein produced a considerable enhancement of the tumors, but not nearly so strikingly and constantly as did the rabbit testicle extract.

Discussion

The experiments here reported show clearly that rabbit testicle extract enhances quite markedly the growth of the Rous sarcoma (Chicken Tumor I), following its injection together with the tumor-producing agent. This enhancement occurs equally well following inoculation of cell-free filtrate of the tumor or with injection of a mash consisting of tumor cells in suspension. In the latter case the resultant tumors appeared much more quickly after inoculation, but the degree of enhancement was essentially the same as that following injection of the filtrate. The tumors arising from tumor mash plus rabbit testicle extract, using the average area of the tumors on the fourteenth day, were 3.37 times the average size of the tumors resulting from injection of tumor mash plus saline. The tumors which resulted from injection of the Berkefeld filtrate plus rabbit testicle extract based on average areas on the thirteenth day were 3.25 times the size of those arising from filtrate plus Locke's solution. Thus it will be seen that the degree of enhancement using rabbit testicle extract was practically the same in two different experiments using tumor mash in one instance and cell-free filtrate in the other.

An interesting finding was the fact that rooster testicle extract prepared under exactly the same condition as rabbit testicle extract was wholly without this enhancing property. Just why this should be true is not clear and we shall not attempt to offer an explanation. However, it was noted at the time of inoculation that the wheals caused by intradermal injections disappeared surprisingly rapidly when rabbit testicle extract was a part of the injection mass, whereas the wheals persisted for a considerable length of time when rooster testicle extract was used.

That normal rabbit serum is not responsible for this enhancement is clearly shown in the second experiment. There was a slight degree of enhancement, as indicated by the average tumor sizes on the thirteenth day. Those tumors which arose from tumor filtrate plus rabbit serum were 1.3 times the size of the Locke's solution control tumors. This is in marked contrast to the enhancement of the tumors by rabbit testicle extract, which not only increased in two plane dimensions, but often protruded and practically always invaded the deep tissues.

It is beyond the scope of this brief communication to attempt to theorize about the mechanism of this remarkable phenomenon. We merely present the experimental facts. For a discussion of this subject, the reader is referred to recent publications by McClean[5] and by Hoffman and Duran-Reynals.[8]

The question naturally arises as to whether this tumor behaves like a true tumor in the sense of mammalian tumors, or whether it is the manifestation of a virus disease, an infectious granulomatous process, or an enzyme-like substance. Without attempting to make any statement which would align us on one side or the other of this controversy, we merely point out the fact that the tumor is greatly enhanced by this agent, which, so far as we can say at present, enhances only virus or bacterial infections. That this tumor is not due to a bacterial agent is pretty well accepted at this time. Enzymes are apparently not enhanced by this substance, and the only experimental work on mammalian tumors thus far reported is that by Duran-Reynals [14] who showed that a rabbit tumor of epithelial origin is actually inhibited by this substance. It is only fair, however, to point out that in the experimental work of Duran-Reynals on the rabbit tumor, the tumor mash plus testicle extract was incubated for from two to three hours at 37° C before injection. This technique was not followed by us. However, our injection masses were made up approximately one to two hours before inoculation and stood at room temperature during that time.

Thus there may or may not be evidence hinting that this tumor falls in the class of diseases due to infectious agents. Before we are definitely willing to say it is so, we should like to see considerable more work done on the effect of this enhancing agent.

Conclusions

1. Rabbit testicle extract markedly enhances growth of the Rous sarcoma (Chicken Tumor I) in chickens.
2. This effect is the same whether tumor mash or a cell-free filtrate of the tumor is used in the inoculations.
3. Rooster testicle extract causes no enhancement.
4. Normal rabbit serum causes a slight degree of enhancement.

BIBLIOGRAPHY

1. Duran-Reynals, F. Exaltation de l'activité du virus vaccinal par les extraits certains organs. *Compt. rend. Soc. de biol.*, 1928, **99**, 6.
2. Duran-Reynals, F. The effect of extracts of certain organs from normal and immunized animals on the infecting power of vaccine virus. *J. Exper. Med.*, 1929, **50**, 327.
3. Duran-Reynals, F., and Suner Pi, J. Exaltation de l'activité du Staphylocoque par les extraits testiculaires. *Compt. rend. Soc. de biol.*, 1928, **99**, 1908.
4. Hoffman, D. C. The effect of testicular extract on filterable viruses. *J. Exper. Med.*, 1931, **53**, 43.
5. McClean, D. The influence of testicular extract on dermal permeability and the response to vaccine virus. *J. Path. & Bact.*, 1930, **33**, 1045.
6. Pijoan, M. The action of testicle, kidney, and spleen extracts on the infective power of bacteria. *J. Exper. Med.*, 1931, **53**, 37.
7. Ledingham, J. C. G., and Barratt, M. M. On the visceral lesions that may accompany experimental vaccinia in rabbits. *Lancet*, 1929, **2**, 515.
8. Hoffman, D. C., and Duran-Reynals, F. The influence of testicle extract on the intradermal spread of injected fluids and particles. *J. Exper. Med.*, 1931, **53**, 387.
9. Carrel, A. Artificial activation of the growth in vitro of connective tissue. *J. Exper. Med.*, 1913, **17**, 14.
10. Drew, A. H. A comparative study of normal and malignant tissues grown in artificial culture. *Brit. J. Exper. Path.*, 1922, **3**, 20.
11. Zakrzewski, Z. Über den Einfluss von Normalgeweben, inbesondere von Geweben endokriner Drüsen, auf das Wachstum von Jensen-Sarkomzellen in vitro. *Ztschr. f. Krebsforsch.*, 1930, **30**, 123.
12. Aievoli, E. Su di un nuovo metodo di innesto chirurgico glandolare. *Riforma med.*, 1890, No. 276, 1652.
13. Rho, F. Testicular extracts in the topical treatment of various ulcers. *J. Trop. Med.*, 1930, **33**, 103.
14. Duran-Reynals, F. The effect of testicle extract and normal serum on the growth of a transplantable epithelial tumor of the rabbit. *Science*, 1930, **72**, 608.

SCIENTIFIC PROCEEDINGS OF THE THIRTY-FIRST ANNUAL MEETING OF THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

CLEVELAND, OHIO

April 2 and 3, 1931

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

ABSTRACT OF BUSINESS SESSION

Voted to elect the following officers for 1931–1932:

President	WARD J. MACNEAL
Vice-President	E. T. BELL
Treasurer	FRANK B. MALLORY
Secretary	HOWARD T. KARSNER
Incoming Member of Council	WILLIAM BOYD

ABSTRACT OF MEETING OF THE COUNCIL

Voted to elect the following new members:

Carl W. Apfelbach,
Granville A. Bennett,
Everett L. Bishop,
Eugene A. Case,
Orren D. Chapman,
Benjamin E. Clarke,
E. V. Cowdry,
Gilbert Dalldorf,
Joseph R. D'Aunoy,
Israel Davidsohn,
Isaac H. Erb,
Sidney Farber,
Clement C. Fenton,
H. W. Ferris,
Donald T. Fraser,
Istvan Gaspar,
Raymond H. Goodale,
Leonard W. Goss,
Allen Graham,
Emmerich von Haam,
Ivan C. Hall,
William B. Hawkins,
C. Alexander Hellwig,
Joseph Kasper,
George J. Kastlin,
Ralph E. Knutti,
K. Robert Koch,
George M. Lawson,
Pablo Morales-Otero,
Lawrence R. Morrison,
E. G. D. Murray,
T. F. Nicholson,
Charles T. Olcott,
Isadore Pilot,
Jacob Rabinovitch,
Nathan Rosenthal,
James F. Rinehart,
George J. Rukstinat,
Helen May Scoville,
Beatrice Carrier Seegal,

George Shanks,
Walter J. Siebert,
Louis Charles Simard,
M. Maxim Steinbach,
Max Strumia,
Kornel L. Terplan,
Richard Thompson,
Thomas T. Walker,
Isabel Mary Wason,
Theodore R. Waugh,
Emil Weiss,
Harry Zimmerman.

Voted to accept the resignations of Dr. O. H. P. Pepper and Dr. Harry C. Solomon.

Voted to record with regret the deaths of Dr. Henry Albert, Dr. C. A. Hamann, Dr. C. A. Krumweide and Dr. R. W. Webster.

Voted to adopt as the topic for the symposium for 1932 the subject of Tuberculosis.

The Secretary drew attention to the difficulties that have to do with selection of titles for the program and it was a general agreement that in the notice of call for the annual meeting it be stated that abstracts must accompany the title cards, drawing to the attention of contributors that wherever possible these abstracts should be prepared for publication with the understanding that if the contributor desires to make any change in the abstract he may do so by providing a substitute abstract before the meeting.

The Secretary drew attention to the demand for publicity on the part of Science Service and newspaper reporters. It was suggested without any definite action being taken that members contributing papers be requested to contribute material suitable for publicity purposes.

The meeting for next year will be held in Philadelphia on April 28 and 29, 1932, which is the week preceding the meetings of the Association of American Physicians in Atlantic City.

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

COMPARISON OF KAHN, SIGMA AND HINTON TESTS. David L. Belding and (by invitation) Julia Arrowood, Boston, Mass.

Abstract. The Kahn, Sigma and Hinton tests were compared in respect to sensitiveness and freedom from variation in the detection of syphilitic reagin. A single lot of high titer syphilitic ascites fluid, which remained stable during the experiment, eliminated any quantitative variation in syphilitic reagin. Graded dilutions of this fluid enabled the results of each test to be expressed quantitatively in arbitrary units. As far as possible, the different antigens were prepared from the same material.

In detecting small amounts of syphilitic reagin, the Hinton test gave a positive reaction with one-half the amount necessary for a positive reading with the Kahn test and one-quarter that for the Sigma test.

The best results were obtained when each test was done with its special antigen. The acetone-insoluble Sigma extract gave similar results to the Kahn-Hinton extract which was not treated with acetone. The Hinton test was effective only with the Hinton antigen. The Kahn and Sigma tests both gave positive results with each other's antigen.

All three tests showed daily variations which appeared to be due largely to the preparation of the antigen dilution. The Hinton test showed the least fluctuation and in this respect would seem to be the most suitable for a quantitative technique.

Discussion

(Dr. Reuben L. Kahn, Ann Arbor.) I would only add that one curve that Dr. Belding showed (wherein there was apparent parallelism between the results of the Wassermann test and those of the modified precipitation tests) seems to indicate that all those tests detect the same substance in syphilitic serum or the same modified condition of the serum that is responsible for these reactions. I believe there is no question at this time that complement fixation and precipitation tests, in spite of variability in their technique, detect the same element or elements in syphilitic serum.

THE ABSENCE OF ANTIGENIC ACTIVITY IN THE COMPLEMENT FIXATION TEST OF THE PURIFIED LIPOIDS, CEPHALIN AND LECITHIN. Elizabeth Maltaner (by invitation), Albany, N. Y.

Abstract. A study of the antigenic activity in the complement fixation test of samples of purified cephalin, free from lecithin, and purified lecithin, free from cephalin, was made. These preparations possessed no antigenic properties either singly or in mixtures, one with the other. Fortification with cholesterol had no effect. The lecithin was lytic in large amounts, but not anticomplementary. The cephalin was not lytic except in relatively large amounts, but was

markedly anticomplementary. Both the lytic and anticomplementary properties were reduced in the presence of serum. The anticomplementary action of cephalin paralleled closely its coagulative activity.

THE QUANTITATIVE DETERMINATION OF THE FIXATION OF COMPLEMENT BY IMMUNE SERUM AND ANTIGEN. Elizabeth Maltaner (by invitation), Albany, N. Y.

Abstract. The conditions essential to quantitative measurement of the changes in hemolytic activity of complement which take place in complement-fixation tests with immune serum were investigated, together with the relations which exist between these changes and the concentration of immune serum.

The previous observations of Morse that the point of 50 per cent hemolysis is the most exact index of the hemolytic unit of complement was applied to this work; also the findings of Brooks which demonstrated that changes in complement activity must be measured at points of equal degree of hemolysis.

The limits of concentration of reagents and the conditions under which quantitative determinations of changes in hemolytic activity could be made were established, using tubercle antigens and their specific antisera. As a result, it became apparent that the variations in fixability of different complements rendered impossible the direct measurement of the immune reaction by use of a hemolytic unit of complement. However, a constant relation was discovered between the extent of the changes in the hemolytic activity of complement and the quantities of immune serum present, which made possible the use of a "fixability unit" in determining the specific activity of serum, instead of the hemolytic unit, thus eliminating the error due to differences in the fixability of different complements.

The observations suggest that by the use of "fixability units," determined with an immune serum selected as a standard, it may be possible to measure quantitatively with different complements the activity of various other sera by comparison.

The development of a quantitative method based upon comparison with a standard immune serum should be an important step toward the standardization of the complement-fixation test, since it would permit the adoption of a specific serum as a universal standard of comparison in evaluating reactions, just as standards of this kind have been adopted in other fields, for example, in comparing toxins and antitoxins.

LOCAL ORGAN HYPERSENSITIVENESS. A NEW METHOD FOR ITS PRODUCTION IN THE RABBIT EYE. Beatrice Carrier Seegal and David Seegal (by invitation), New York City.

Abstract. The observations of Auer, Opie, Hanger and Menken have shown that when a local area of the body is subjected to a sterile inflammation, a foreign dye or protein circulating in the blood is concentrated in this area of local inflammation. Utilizing this principle, we have been able to show that if a rabbit's eye is inflamed and egg white is injected intravenously at the same time, the egg white is concentrated at the site of this inflammation in sufficient quantity to render this eye sensitive to subsequent intravenous injection of the homologous antigen.

The experiments were carried out on three groups of animals. Group I were the experimental animals, Groups II and III the control animals. Group I re-

ceived 0.15 cc. of glycerine into the anterior chamber of each right eye under cocaine anesthesia. The glycerine, which contained no protein by the Biuret test, produced a violent sterile inflammation in the eyes, lasting several days. Three intravenous injections of 2 cc. of a 20 per cent solution of fresh egg white were given to each of the animals of this group on the same day as the glycerine injection of the eyes and on the two succeeding days. Animals of Group II received only glycerine into their anterior chambers, but no egg white intravenously, while the third group of animals received only the three injections of egg white intravenously, but no glycerine into the eyes. Three weeks later, when all eyes had returned to a normal appearance, the animals of these three groups received 1 or 2 cc. of a 20 per cent solution of fresh egg white intravenously. In one to two hours the injected eyes of Group I, which had been prepared by intravenous injections of egg white associated with an injection of glycerine into the eye, responded with marked hyperemia of the iris and conjunctiva, lacrimation and edema of the lower conjunctival sac. The animals which received glycerine only into the anterior chamber and those animals which received only egg white intravenously did not respond to this shocking injection of egg white. The accompanying table summarizes these experiments.

	Group I Glycerine in right eye and egg white intravenously	Group II Glycerine in right eye	Group III Egg white intravenously
Three weeks later. Egg white intravenously.	Right eye: hyperemia of conjunctiva and iris; lacrimation; edema.	No response	No response

The sensitization produced by this indirect method of injury to the eye associated with intravenous injection of the antigen was similar to the sensitization which we have previously reported following direct injection of the antigen into the anterior chamber. The reaction occurring after the intravenous shocking injection of egg-white appeared in the same time interval of one to two hours, lasted for a similar period of about twenty-four hours, and proved to be specific for egg white. The reaction could be repeatedly elicited by the properly spaced injections of the antigen.

The occurrence of a simultaneous hyperemia in the opposite uninjected eye during the reaction following the shocking injection of antigen was noted frequently in this series of animals. This reaction was at most about half as intense as that in the prepared eye. Woods and Riehm have each reported somewhat similar observations in their studies in sympathetic ophthalmia. They sensitized one eye by direct injection of the antigen and found that later parenteral injection of the antigen produced a bilateral reaction. We have occasionally seen a slight reaction in the contralateral eye in our previous series of experiments, but not as marked as in this group of animals. As yet, we are unable to interpret the reactions in the contralateral eye.

In conclusion, therefore, the observation that an inflamed area of the body becomes more richly saturated with a circulating foreign protein than does neighboring normal tissue has been utilized to produce specific active local sensitization of the rabbit eye.

Discussion

(Dr. Marcus W. Lyon, South Bend.) This phenomenon seems to be correlated with a clinical phenomenon in diseases of the skin. A diseased area of the skin which has been irritated by one substance at one time, subsequently is much more susceptible to toxic substances other than the specific one. That observation has been known to clinicians for a great many years, and a few days before I left I had a clinician call my attention to that interesting phenomenon.

(Dr. Charles Weiss, St. Louis.) I should like to ask if, in the course of examination of the corneas of animals during which you recorded keratitis, you at any time observed pannus.

(Dr. Seegal, closing.) We did very rarely observe pannus.

LOCAL ORGAN HYPERSENSITIVENESS. THE PRODUCTION OF ACTIVE HYPERSENSITIVENESS IN THE RABBIT EYE BY STREPTOCOCCUS SCARLATINAE AND ITS PRODUCTS. David Seegal and Beatrice Carrier Seegal (by invitation), New York City.

Abstract. It was found that *Streptococcus scarlatinae* filtrate when injected into the rabbit's anterior chamber conferred a specific hypersensitivity in that organ so that the parenteral injection of only the *Streptococcus scarlatinae* or a *Streptococcus erysipelatis* filtrate was able to reactivate the sensitized eye. The typical eye reaction could be elicited not only by the parenteral injection of the *Streptococcus scarlatinae* filtrate, but also by the same *Streptococcus scarlatinae* organism living and growing in the subcutaneous tissue of the rabbit. This was accomplished by producing a nidus of infection in the form of an inoculated agar gelatine plaque first described by Dochez. The eye reaction appeared in one to three hours after the injection of the mixture. Plaques containing heat-killed organisms produced no eye reactions. It was further found that the typical eye reaction could be prevented if neutralizing amounts of *Streptococcus scarlatinae* antiserum were injected prior to the administration of the shocking dose of filtrate. A filtrate of a non-scarlatinal *Streptococcus hemolyticus* and a *Staphylococcus aureus* filtrate were unable to relight these eyes.

These experiments seemed to offer evidence that the filtrate of *Streptococcus scarlatinae* could confer a specific sensitivity in the rabbit eye, that the shocking qualities of the homologous filtrate could be elaborated in the distant tissues of the animal and that this agent could be specifically neutralized.

Although the eyes sensitized to the *Streptococcus scarlatinae* filtrate could not be relit by the parenteral administration of a large number of agents, such as egg white, guinea pig red cells and nucleoprotein fractions of the *Streptococcus scarlatinae* bacterial body, it was repeatedly found that the Streptococcus scarlatinal filtrate was able to produce the typical eye reaction in rabbits in whose anterior chamber such substances as beef broth, Streptococcus scarlatinal bacterial body nucleoproteins, egg white and glycerine had been injected. These results immediately implied that although *Streptococcus scarlatinae* filtrate could confer a specific hypersensitivity in the rabbit anterior chamber, it also possessed a remarkable ability to cause a non-specific relighting of eyes injected with a large number of heterologous substances. It was at first considered that a simple injury phenomenon originally induced in the eye was the cause of the subsequent relighting process. This might have explained the ability of the eye injected with glycerine to respond to the intravenous injection of the *Streptococcus scarlatinae*

filtrate, for the glycerine had no protein in it by the Biuret test. However, eyes injured with saline, iodine and albolene failed to respond to the subsequent injection of the *Streptococcus scarlatinae* filtrate.

It was apparent that the agent in the *Streptococcus scarlatinae* filtrate effective in causing the non-specific relighting of the eyes must in all probability be the toxallergin, since this reaction could be specifically neutralized by the *Streptococcus scarlatinae* antiserum. Furthermore, this ability of the *Streptococcus scarlatinae* filtrate to reactive eyes in which these heterologous substances had previously been injected did not appear to be common to several other filtrates studied by us. For instance, a non-scarlatinal *Streptococcus hemolyticus* filtrate and a *Staphylococcus aureus* filtrate were ineffective in producing the eye reactions in these animals. Other filtrates, however, must be tried before conclusions can be drawn on this point. The crucial point is the fact that whereas the *Streptococcus scarlatinae* filtrate is able to induce in the rabbit eye such a specific hypersensitiveness that only the homologous or the *Streptococcus erysipelatis* filtrate will incite the characteristic eye reaction; this same filtrate when injected intravenously is able to relight eyes in whose anterior chamber such varied substances as glycerine, broth, egg white and guinea pig red cells have been introduced weeks or months previously.

Discussion

(Dr. Augustus B. Wadsworth, Albany.) I am very much interested in these reactions and their specific character. The statement that the "scarlet fever" and "erysipelas" organisms were the only ones that reacted specifically, and that the antiserum neutralized this action, is of interest to me. I think it is important, however, to emphasize the fact that any distinction between the streptococci from scarlet fever used in such experiments, and those from erysipelas, depends greatly upon the selection of the strains used. Some of the strains of erysipelas streptococci (I speak of them as "erysipelas" streptococci, not because it is possible to distinguish any particular group of streptococci associated with any particular pathological process by any known means, but simply because the organisms are isolated from these conditions) correspond in their toxic properties with some of the streptococci from scarlet fever. Others do not, and the experiments should be very fully controlled with different streptococcic toxins. The strains from scarlet fever vary in their toxin production. The New York 5 is perhaps one of the broadest strains. When its serum was tested with the toxins of 500 different cultures, between 70 and 80 per cent corresponded. Some of the streptococcus toxins of cultures from erysipelas and from scarlet fever are not neutralized by the antitoxin of the New York 5, whereas others are; also the valency of the toxin as determined by the toxin-antitoxin neutralization with homologous sera varies enormously. In our experience we have only met one or two strains of the broad valency and antigenic action of the New York 5. I think one must control the specificity of the reaction in all such experiments by the accurate determination of the homologous and heterologous action of representative sera in each instance.

(Dr. Max Pinner, Tucson.) The experiments reported may go a long way to explain the very frequent failure to elicit a skin reaction with a specific allergen which apparently brings about the allergic manifestations of disease. I might quote a personal experience which is probably a good example in question.

Some years ago, following a rather extensive sinus operation, I worked a good deal with dried spinach leaves and in the course of about three weeks I had rather violent hay fever attacks every time I came in contact with the leaves. Before that time I had never had any allergic phenomena. At that time no skin reaction could be elicited by a saline suspension of the leaves, which on inhalation would immediately elicit violent attacks. Anyone who has done skin tests on patients with undoubted allergic phenomena has found probably a considerable percentage of patients who will not give the expected skin reaction to the injections, but who on spontaneous administration, as it were, will regularly have an attack. The experiments which were reported, I think, give a very convincing explanation for such a fact, namely that the general assumption is probably not correct that in allergic disease the whole body is prepared to react. It may be only a localized area or one special organ which is sensitized to react with allergic manifestations as soon as the specific allergen reaches the sensitized tissue in sufficient concentration.

PHENOMENON OF LOCAL SKIN-REACTIVITY TO BACTERIAL FILTRATES: PASSIVE IMMUNITY TO REACTING FACTORS. Gregory Shwartzman, New York City.

Abstract. It is possible to elicit passive immunity to *B. typhosus* reacting factors by means of normal and immune homologous neutralizing bodies. The "in vivo" serum protection against these factors follows the law of multiple proportions.

Passive immunity is best obtained when the immune serum is injected intravenously one-half hour before the intravenous injection of the reacting factors.

It is also possible to prevent the occurrence of the local skin reaction by an intravenous injection of serum after the intravenous injection of the reacting factors, provided that the serum dose is very large and the serum injection is made immediately after the filtrate injection.

The results also demonstrate passive serum protection against the lethal effect of *B. typhosus* "agar washings" filtrates, suggestively, in multiple proportions.

Discussion

(Dr. Augustus B. Wadsworth, Albany.) Dr. Shwartzman has made such an important contribution that he deserves to be congratulated. For the past year or two we have followed his work very closely, particularly with antimeningococcus serum. His methods I am sure will be of considerable value as a check or control on the activity of sera used in the treatment of infections, the inciting organisms of which do not produce a sufficiently potent toxin or lack virulence to kill animals, so that a protection test can be used. It is an important method and should be given a thorough trial. I am particularly interested in the mechanism of the lesion, and I wanted to ask Dr. Shwartzman if he would explain his conception of the mechanism of the pathological process in the cutaneous lesion. I have wondered if the process giving rise to the varying degrees of reaction might not be thrombosis leading to the reaction of hemorrhage, so that the extent of the thrombosis rather than the direct action of the toxin might account for some of the variation in the local lesion. I have not had a chance to study many of the sections, and knowing that Dr. Shwartzman has, I should be interested in his interpretation of the pathogenesis of the local lesion.

(Dr. Shwartzman.) I shall have to answer Dr. Wadsworth's question by saying that we have not as yet made a sufficient histological study of the lesions. The most obscure thing about the phenomenon is that the second injection has to be given intravenously. One can reinject the same area of the skin with as much as 100 times the amount necessary and not get any reaction. Nevertheless a very minute amount injected intravenously may produce a severe necrosis. I was able to obtain a reaction with a dilution of the meningococcus filtrate as high as 1:2500. I understand that Drs. Wadsworth and Sickles obtained reactions with still higher dilutions. If you consider the fact that this small amount is still further diluted in the blood stream about 100 times and that some of it will be lost in the organs before reaching the prepared skin area, the amount which will actually produce the lesion must be amazingly small. I believe that the fact that the filtrate is only active when introduced through the intravenous channel is intimately related to the mechanism and pathogenesis of this phenomenon. I am not prepared to say just where the first injury in the prepared area takes place, but I am under the impression that the thrombosis is secondary, and that the primary effect is on the endothelial lining of the blood vessels. Dr. Klemperer is conducting some investigations on this point.

(Dr. Ernest A. Pribram, Chicago.) I wish to say that a high dilution of an antitoxin acts in a more delayed fashion than a lower dilution, and this phenomenon has also been observed in diphtheria antitoxin by Hamburger. He also recommended using diphtheria antitoxin in a diluted form. It was an improvement in technique to dilute the serum first and inject it in the diluted form. The sera which are on the market at the present time are usually diluted after the removal of albumin.

MODIFICATION OF THERAPEUTIC SERA WITH A VIEW TO AVOIDING COMPLICATIONS OF ALLERGIC NATURE. J. Bronfenbrenner and (by invitation) Donald M. Hetler and I. O. Eagle, St. Louis, Mo.

Abstract. Chemical modification of therapeutic sera according to methods initiated by Obermeier and Pick and by Landsteiner has yielded products which retain a considerable proportion of original antibodies, while they have lost the species specificity of the carrier serum. These products can be administered to animals highly sensitive to horse protein without causing any untoward symptoms. With respect to their antigenic properties many of these preparations are mutually heterologous, thus permitting their use one after another without allergic manifestations.

Discussion

(H. M. Powell, Indianapolis, by invitation.) I believe that this line of work is of importance to commercial producers of antitoxin; it appeals to the imagination, and would seem to offer possibilities of a whole series of serums which might be arranged in such a fashion that no one individual would ever need to receive two of the same kind of diazotized serums, and it would obviate the possibility of individuals receiving antiserum which was not completely dehorsed.

(Dr. Augustus B. Wadsworth, Albany.) I did not quite understand the percentage of loss of potency after treatment.

(Dr. R. S. Muckenfuss, St. Louis.) I am not familiar with the figures as I am presenting the paper for Dr. Bronfenbrenner, but I understand it did vary with

different preparations, and there were one or two in which there was no loss of potency.

(Dr. Stuart Mudd, Philadelphia.) May I call attention to another phase of this very important procedure on which Dr. Reiner has already published a preliminary paper, namely the possibility of making "charmed bullets" by coupling a substance toxic for a bacterium to the antibody, which will unite the toxic substance with its "target." I suggest that this possibility be kept in mind in the future development of this type of work.

STUDIES ON THE PROPERTIES OF BOUILLON FILTRATES OF THE MENINGOCOCCUS. N. S. Ferry, Detroit, J. F. Norton, Detroit, and A. H. Steele (by invitation), Northville, Mich.

Abstract. The meningococcus, when cultivated in hormone broth, exhibits within a few days a luxuriant growth with a peculiar heavy pellicle, and produces in the broth filtrate a toxic substance with properties characteristic of a soluble toxin.

Filtrates from bouillon cultures of the four recognized types of the organism contain toxins apparently type specific, although there appears to be a toxin present in the filtrates common to all types. These filtrates, in high dilutions, produce local skin reactions in a certain number of individuals, depending upon the original strength of the toxin, and whether it is modified by heat or age.

The toxins of the four types, either individually or mixed, when injected into animals, stimulate the formation of antitoxins and these in turn neutralize the homologous toxins in fairly high dilutions. The toxins are also neutralized by meningococcus meningitis convalescent sera and the action is apparently specific to a certain degree.

Lytic extracts of the meningococcus do not act in a manner similar to the bouillon filtrates, supporting the view that the toxins in the filtrates are extracellular in nature.

It is concluded that the various types of the meningococcus produce extracellular toxins in bouillon cultures and that these in turn can stimulate the development of specific antitoxins.

Discussion

(Dr. Gregory Shwartzman, New York City.) This paper is very interesting to me especially, because of some similarities between the human skin test which the authors reported and the work which I have done on the phenomenon of local skin reactivity to meningococcus filtrates. The first point is the fact that Type 3 meningococcus yields filtrates of higher potency than Type 1. In my work I find that Type 3 meningococcus produces filtrates from two to five times stronger than Type 1. The second point of similarity is the fact that autolyzed bacteria contain practically no toxic substances for either test. I should like to ask Dr. Ferry whether he observed any swelling at the site of the injection similar to that observed in rabbits by Dr. Ecker.

(Dr. Ferry, closing.) As far as I have been able to determine, the toxin from Type 3 in general is no stronger than the toxin from the other three types. This individual Type 3 culture is a rather recently isolated strain and produces a toxin much stronger than any other Type 3 strain which has been growing much longer under artificial culture.

In regard to the question of this filtrate and the reaction which Dr. Shwartzman is getting with his agar washings, I feel compelled to refer to the work

which I carried out several years previously on agar washings, with my associate, Dr. Fisher. The original agar washings were prepared exactly as Dr. Shwartzman's filtrates and at that time we did not consider them very toxic, although they were used for diagnostic skin reactions. As far as I can judge, the toxic material from the bouillon filtrates is entirely different from anything found in the agar washings. The bouillon filtrate contains a soluble toxin, and I feel pretty sure that the agar washings do not. A specific antigen is present in the washings and it may be toxic, but I cannot consider it as a soluble toxin.

In regard to the reaction at the site of injections in the human, as mentioned by Dr. Shwartzman, we have not noticed any swellings similar to those described in rabbits by Dr. Ecker.

A Study of Antipneumococcus Preparations Before and After Drying. K. George Falk, Eugenia Valentine, Grace McGuire, and Elinor Whitney (by invitation), New York City.

Abstract. A method of drying antipneumococcus serum *in vacuo* was described whereby the antibody property was retained intact and could be redissolved. After storage in the icebox for six to twelve months the powdered antiserums were the same, or only slightly lower than the homologous untreated serums in their mouse protective units, agglutinin and precipitin titers and in the amount of precipitate with soluble specific substance (Type 1).

When stored at room temperature, on the other hand, the agglutinin and precipitin content had almost completely disappeared and no precipitate was formed with a 1:10,000 dilution of soluble specific substance (Heidelberger), but the mouse protective units had fallen only one-half at the most.

Neutralization or Destruction of Diphtheria Toxin by Tissue. Augustus B. Wadsworth and (by invitation), Ella N. Hoppe, Albany, N. Y.

Abstract. The action on diptheria toxin of the living animal tissues was studied by using tissue culture methods *in vitro*, and supplementing those with *in vivo* inoculations, for the quantitative determinations. Guinea pigs, guinea pig tissues, and media entirely of guinea pig origin were used throughout the work. Diphtheria toxin was not altered by contact with fetal or adult cardiac tissue in a state of survival. It lost its toxicity if the tissue was growing in a state of cultivation. The tissues were apparently uninjured by the toxin in the dilutions used, and continued to grow uninterruptedly.

Discussion

(Dr. Bezi, Rochester, N. Y.) What dose of diphtheria toxin was neutralized by guinea pig embryo cardiac tissue?

(Dr. Wadsworth.) 1: 500 MLD in 0.1 cc.

(Dr. N. Popoff, Rochester, N. Y.) Did you try as a control the effect of the monocytes, and an extract of the erythrocytes on the toxin? I ask this question in connection with some work done on the detoxicating influence of an extract of red cells upon toxins.

(Dr. Wadsworth, closing.) In the early days we used only leucocytes, and we have tried from time to time to get a differentiation of these cells — the monocytes. The experiments in the last year have been directed particularly to an attempt to sustain these cells in an active growing state free from other cells, but we

do not feel that we have accomplished it, so we are continuing the experiments with different tissues. We have not yet been able to do the experiments you suggest.

FURTHER OBSERVATIONS ON A SKIN TEST FOR SUSCEPTIBILITY TO POLIOMYELITIS. Edward C. Rosenow, Rochester, Minn.

Abstract. The report consists of a brief presentation of the method used in preparing suitable antigens from the streptococcus isolated, of results of skin tests in persons at the onset of attacks of poliomyelitis, during convalescence and after recovery, and in well persons of different age groups who have not had poliomyelitis, and of the neutralizing power of the serum of "skin test positive" and "skin test negative" individuals.

THE IMMUNIZATION OF MONKEYS AGAINST THE VIRUS OF POLIOMYELITIS. William B. Brebner (by invitation), St. Louis, Mo.

Abstract. This report deals briefly with experiments showing that many infective doses of poliomyelitis virus may be injected into the spleen of normal monkeys with little risk of the host developing any symptoms of the disease. A short time after the splenic injections it may be shown by neutralization tests that the animals have acquired an appreciable degree of immunity. The significance of these observations as concerns the virus disease group as a whole is considered and mention is made of the importance of "placing" a virus antigen.

Discussion

(Dr. E. C. Rosenow, Rochester, Minn.) I am not quite certain regarding the method of injection of the virus. I understood you to say that both injections were made into the spleen.

(Dr. Brebner.) In all cases the virus was given directly into the spleen.

(Dr. Rosenow.) Was there a rise in temperature following the first injection or following the second injection?

(Dr. Brebner, closing.) The second time that the virus was injected intrasplenically there was a higher temperature of a plateau type, but no symptoms of paralysis. This reaction, in Jungeblut's opinion, is evidence of a sensitization.

SOME IMMUNOLOGICAL ASPECTS OF LEUKEMIA. W. C. Hueper and (by invitation) M. Russell, Philadelphia, Pa.

Abstract. The high increase of leucocytes in the blood of leukemic patients is not caused by a decrease or absence of the normal antiproliferative action of blood serum upon leucocytes, because it was found that in tissue cultures of normal leucocytes in leukemic plasma this quality was rather enhanced than decreased, if compared with that of normal plasma.

Antileucocytic serum produced by repeated intravenous injection of leukemic leucocytes into rabbits is non-specific in its action against leucocytes, as also the growth of normal leucocytes is completely inhibited by the use of antileucocytic plasma in tissue cultures. The antiserum does not contain any leucolysins. The antiproliferative titer of such sera to be used for therapeutic purposes in leukemias can be determined by the method of leucocytic tissue cultures.

THE EFFECT OF SUPRARENAL CORTICAL EXTRACT ON RESISTANCE. David Perla and J. Marmorston-Gottesman, New York City.

Abstract. Extract of the cortex of suprarenal gland prepared according to the method of F. A. Hartman is a highly potent extract. It is free of toxicity and epinephrin. Injections of cortin will raise the resistance of suprarenalectomized rats to several lethal doses of typhoid vaccine and to several lethal doses of histamine or ergamine acid phosphate for suprarenalectomized rats. The protective action of cortical extract against poisoning with typhoid or histamine in suprarenalectomized rats may be utilized as a means of biologically assaying the potency of such extracts. The amount of cortical extract injected intraperitoneally into suprarenalectomized albino rats on the fifth and sixth day after operation necessary to protect these rats against 200 mg. of ergamine acid phosphate per Kg. of body weight may be considered a standard unit.

STUDIES ON AN INSTITUTIONAL OUTBREAK OF POLIOMYELITIS APPARENTLY DUE TO MILK. Edward C. Rosenow, Rochester, Minn.

Abstract. The report consists of a brief statement of methods used and results obtained in a study of the cases that occurred; of the streptococcus isolated from throats and spinal fluid of patients; of the throats of the student body; of the milk from the cows at the dairy supplying the milk; of skin tests at the time of the epidemic and six weeks later at the affected college; and of control studies at a college in the same town where no cases occurred.

EXPERIMENTS RELATIVE TO A POSSIBLE BASIS FOR VACCINE THERAPY IN RHEUMATIC FEVER. B. J. Clawson, Minneapolis, Minn.

Abstract. Patients having acute rheumatic fever have been shown to be hypersensitive (allergic) to streptococcic protein.

Animals can be made hypersensitive to streptococci so that lesions will result from later injections with much smaller doses than in normal animals.

The cellular reaction in small lesions in the hypersensitive animal is similar to that found in tissues in human rheumatic infections. There is a marked similarity in the hypersensitiveness (allergy) to streptococci in animals made hypersensitive experimentally and in patients having acute rheumatic fever.

Rabbits made hypersensitive to streptococci can be desensitized by intravenous administration of a streptococcic vaccine which does not have to be type specific.

The results of these experiments suggest the use of intravenous vaccine treatment in acute rheumatic fever.

BACTERIAL PLEOMORPHISM: THE DERIVATION OF NON-VIRULENT STAPHYLOCOCCUS ALBUS FROM VIRULENT STAPHYLOCOCCUS AUREUS. Max Pinner and (by invitation) Marie Voldrich, Tucson, Ariz.

Abstract. The relation between *Staphylococcus aureus* and *Staphylococcus albus* is essentially the same as that between S and R strains of other bacterial species.

1. Albus splits off spontaneously from pure line strains of aureus.

2. This splitting off can be hastened and increased by culturing aureus in serum containing aureus agglutinins.

3. From albus strains, aureus colonies can be isolated by culturing albus in anti-albus serum.

4. The following differences have been established between aureus and albus:
(*a*) Aureus is virulent for rabbits, albus is not.
(*b*) The two strains produce strain-specific agglutinins, which are specifically absorbed only by the homologous strain.
(*c*) Aureus is an active proteolyzer, albus is not.
(*d*) Aureus coagulates citrated blood plasma, albus does not.
(*e*) Aureus produces a hemolysin, albus does not.
(*f*) Aureus produces a higher degree of alkalinity in beef broth infusion than does albus.

5. Rabbits can be rendered completely immune against aureus by injecting them with living albus.

6. Whereas pigment production, virulence and agglutinability go strictly parallel, and are, therefore, dependent on environmental conditions, it was found that the susceptibility to phage remains the same for each strain regardless of its dissociative changes.

7. It is of interest to point out that in staphylococci the phenomenon of dissociation does not involve the usual change in colonial morphology — smooth and rough — but an alteration of pigment production.

BLOOD CHEMICAL CHANGES IN EXPERIMENTAL STREPTOCOCCUS SEPTICEMIA. Richard W. Linton (by invitation), New York City.

Abstract. When rabbits are given fatal doses of *Streptococcus hemolyticus* intravenously it is found that some die with fulminating infections within thirty-six to seventy-two hours, and others, which are designated as having acute infections, survive on the average six days.

The following changes have been found to occur in the blood of these animals:

The blood sugar concentration drops at a constant rate throughout the disease, but does not reach a condition of hypoglycemia.

Glycogen is present in the liver at death.

The CO_2 capacity is lowered markedly at first, then returns to a somewhat higher although still subnormal level, at which it continues until the terminal stages of the disease, when the acidosis becomes very marked.

Inorganic phosphorus is markedly increased in concentration at the terminus of the disease. This increase is greater in animals showing an acute course than in those in which the disease is of the fulminating type.

Calcium also shows terminal changes, decreases occurring in the animals with fulminating infections to a greater degree than in those with acute infections. In both, abnormally low levels are reached.

Non-protein nitrogen and creatinine are greatly increased in the terminal stages, in both groups of animals.

It is considered that the results obtained may be explicable on the assumption of a large amount of acid production *in vivo* by the streptococcus. The resulting acidosis becomes uncompensated in the terminal stages of the infection, thus leading to severe changes in the tissues. In the kidney these take the form of an acute diffuse nephritis, which is sufficient in degree to bring about the death of the animal.

SPONTANEOUS INSULIN RESISTANCE IN RABBITS: THE EFFECT OF THYROIDECTOMY ON THE RESPONSE TO INSULIN. Isolde T. Zeckwer, Philadelphia, Pa.

Abstract. Two rabbits were found showing spontaneous resistance to enormous doses of insulin. One failed to develop convulsions with doses of insulin up to

66 units, but went into convulsions with 76 units. The second rabbit required 20 units for development of convulsions. With large subconvulsive doses, the blood sugar remained for many hours near the convulsive level, but apparently the release of glycogen from the liver was sufficient to maintain the blood sugar just above the critical level. During this period, there was frequently exophthalmos, indicating increased activity of the sympathetic nervous system.

In view of the work of Bodansky, of Burn and Mark, and of Britton and Myers, showing that thyroidectomy increased the sensitivity of normal animals to insulin, it was determined to ascertain whether the thyroid was responsible for the abnormal resistance of these two rabbits.

Thyroidectomy was performed under aseptic precautions. The animals became increasingly responsive to insulin as days elapsed after operation, although gaining in body weight. By the twentieth postoperative day, both developed convulsions after 5 units.

The gross and microscopic appearance of the excised thyroids showed no hyperplasia or pathological change.

Apparently the thyroid, although anatomically normal, was functionally responsible for increased sensitivity of the sympathetic nervous system which permitted such ready sympathetic discharge of glycogen from the liver that the blood sugar level could be maintained above the convulsive level after enormous doses of insulin.

CERTAIN PHYSICO-CHEMICAL PROPERTIES OF BLOOD AND EXUDATIVE CELLS. Stuart Mudd and (by invitation) Emily B. H. Mudd, Philadelphia, Pa.

Abstract. Application of the interfacial technique permits the study of the wetting properties of cells and of their behavior under compressing and stretching forces. Polymorphonuclear leucocytes are cells of relatively low viscosity, the majority of which disintegrate under the tension of an oil-water interface. The large mononuclears are cells of higher viscosity, the majority of which are comparatively little deformed by the interfacial tension. Lymphocytes and various leucemic cells vary within these limits. All leucocytes studied have *hydrophilic* surfaces, and are strongly resistant to wetting by oil.

Erythrocytes have visible surface membranes, which are capable of withstanding tension. The surfaces of erythrocytes are relatively *hydrophobic*, and offer little resistance to wetting by oil.

The interfacial tension relations at the surfaces of the cells in an oil-water boundary have been analyzed.

Discussion

(Dr. Ernest A. Pribram, Chicago.) It would be very interesting to study the physico-chemical differences between the red blood corpuscles and the leucocytes which cause their different reaction to the oil. The red blood corpuscles are positively charged, as they contain the highly positively charged iron. The monocytes have a large basophilic, *i. e.*, negatively charged nucleus and a relatively small protoplasm without granules. The granulocytes or polymorphonucleated cells have a relatively small nucleus and a large protoplasm, which contain neutrophilic, *i. e.*, positive and negative or amphoteric granules. Their electronic position is therefore in between the erythrocytes and monocytes. There are further phosphates present in the leucocytes and monocytes, sodium and potassium salts, which may diffuse through the medium to the oil. They

may combine with the oil and saponify the oil, as substances which lower the surface tension of a colloid concentrate on its surface. So I should like to ask two questions: Is there a difference in the absorption of substances by the oil (in an aqueous medium), if these substances have a different electric charge and if they contain different diffusible substances, such as the phosphates, which may combine with the oil?

(Dr. Mudd, closing.) If one could introduce an electrode into a red cell and measure the total potential difference (thermodynamic potential difference) between the inside and outside of the cell, possibly the red cell might be positive, although I know of no such measurements. The basophilic and acidophilic nature of the substances in the cell depends upon their dissociation state. I am not discussing either of these things, but am discussing the surface properties of the red cell. The surface charge of the red cell relative to its suspending medium (electrokinetic potential difference) is negative. Any readily adsorbable impurities present in the suspending medium may alter the surface properties of the cell, and errors due to such adsorption effects should be carefully guarded against.

FIXATION OF BACTERIA AND OF PARTICULATE MATTER AT THE SITE OF INFLAMMATION. Valy Menkin, Boston, Mass.

Abstract. India ink or graphite particles injected into an area of inflammation fail to disseminate to the tributary lymph nodes. When injected into a normal peritoneal cavity they rapidly appear in the retrosternal lymph nodes. When injected into an inflamed peritoneal cavity they are fixed *in situ* and fail to reach the regional lymph nodes.

Graphite particles injected in the circulating blood stream enter an inflamed area both as free particles owing to the increased capillary permeability, and also as phagocytosed material within leucocytes.

Bacteria (*B. prodigiosus*) injected into inflamed tissue are fixed at the site of inflammation and fail to disseminate to the regional lymph nodes as readily as when injected into normal tissue.

Bacteria (*B. prodigiosus*) injected at the periphery of an inflamed area do not readily penetrate into the site of inflammation. The experiments furnish evidence, in addition to that already provided in previous work, that fixation of foreign substances by the inflammatory reaction is primarily due to mechanical obstruction caused by a network of fibrin and by thrombosed lymphatics at the site of inflammation. Fixation occurs very early in the development of the inflammatory reaction (shown in some previous experiments to take place as early as thirty minutes after the injection of the inflammatory irritant). The rapid formation of a fibrin network and of thrombi in lymphatics at the site of inflammation circumscribes the irritating substance and thus prevents its passage into the blood stream. This allows of a definite interval of time for the leucocytes to assemble for phagocytosis. The initial fixation of bacteria or of other injurious substances at the site of inflammation thus becomes a protective mechanism and plays a definite rôle in immunity.

Bacteria (*B. prodigiosus* and *B. pyocyaneus*) injected intravenously rapidly enter an inflamed area. It is suggested that localization of bacteria in a *locus minoris resistentiae* may be explained as the result of increased capillary permeability with subsequent accumulation and fixation of bacteria from the blood stream at the point of injury.

TULAREMIC ENCEPHALITIS: PATHOLOGY OF ACUTE TULAREMIA WITH ENCEPHALITIS AND COEXISTING ACTIVE TUBERCULOSIS. F. W. Hartman, Detroit, Mich.

Abstract. Gross and microscopic findings in the body of a patient who gave the history of being infected while working in a butcher shop, followed by ulceration at the base of the thumb on the right hand, lymphangitis of the arm and axillary adenitis, then several weeks' course in the hospital, which was characterized by fever and semidelirium, are given. Serum agglutination was shown with *B. tularense* 1: 2500. Grossly characteristic areas of necrosis were found throughout the lungs, lymph nodes and spleen. The brain showed areas of necrosis in the corpus callosum and basal nuclei. *B. tularense* were demonstrated in sections of these tissues. The left kidney showed characteristic ulcerative tuberculosis in the calyces, and the right epididymis showed an old tuberculous abscess. Tubercle bacilli were demonstrated in these latter tissues in abundance.

Discussion

(Major George R. Callender, Washington.) I understand there were no lesions found in the meninges.

(Dr. Hartman.) No.

(Major Callender.) There is one case reported in which the history was typical and the autopsy showed lesions in the meninges, but none in the brain. These lesions I believe were due to *B. tularense*, but as no agglutination was done, it is not a proved case.

TULAREMIC PNEUMONIA. Howard H. Permar and W. W. G. MacLachlan, Pittsburgh, Pa.

Abstract. A histopathological study of a case of acutely fatal tularemia showing an acute diffuse tularemic infection of the lung, a tularemic pneumonia, is presented. Death occurred on the seventeenth day. The clinical course was characterized by extreme toxicity. Consolidation developed in the last few days of life. The autopsy is the ninth reported on tularemia, and the fifth showing a diffuse acute pneumonia at autopsy. The lesion is an interstitial and alveolar pneumonia, with a mononuclear cell response. There are typical miliary necrotic nodules in the interstitial tissues about bronchi and vessels, like those seen in liver and spleen in animals, and in very acute human tularemic infections. The alveolar exudate tends to necrose; and because of a peculiar inflammation of the vessels with narrowing and thrombosis, large areas of the lung tissue become necrotic. The finer air passages are involved, like the alveoli, following the interstitial inflammation. The process is not bronchiogenic.

Discussion

(Dr. Alfred Plaut, New York City.) Have attempts been made to find the organisms in the sections, and secondly, is the lesion in the blood vessel not merely a proliferation of the intima itself?

(Dr. Permar, closing.) I believe nobody has ever found organisms in the human tissues. It is possible to find them in experimental animals if the experimental infection has been rapidly fatal. We tried the Giemsa and other stains, and could not find them.

Of course we looked for lesions in the intima, because that is the striking lesion in tularemia in the walls of the granulomatous abscesses, and I believe it is largely a process of edema and cellular infiltration, and not a true proliferative change in the lung at this stage.

A Study of Vaccine Virus Pneumonia in Rabbits. R. S. Muckenfuss, H. A. McCordock and (by invitation) S. J. Harter, St. Louis, Mo.

Abstract. A strain of the neurovirus of Levaditi, furnished by Dr. Rivers, and carried by testicular passage, has been used in these experiments. The diluted testicular extract was injected into the lungs by passing a needle through the skin and anterior tracheal wall, or by inserting a catheter through the mouth into the trachea. Thirty rabbits, both normal and vaccine immune, have been studied. After inoculation most of the animals appeared to remain well. Temperature changes were so rare as to be of no significance. Frothy fluid flowed from the nose of a few rabbits that died, if they were held downwards.

The virus incites a characteristic pneumonia in normal rabbits which can be recognized as early as forty-eight hours. In typical cases there are grayish brown to dark red patches of gelatinous consolidation in both lungs, though often more marked in one, and usually situated in the lower lobes, posteriorly. The mucous membrane of the trachea and primary bronchi is hyperemic. If a catheter has been used there is no local reaction. After the use of a needle animals show a marked edematous swelling of the loose connective tissue of the neck, and occasionally of the mediastinum. These animals always show an intense inflammation of the tracheal mucosa at the site of injection.

Microscopically, groups of alveoli are filled with coagulated albuminous exudate. In places this acute edema is the only change seen. Some of the alveoli contain large masses of tangled fibrin; others have a few isolated threads mixed with the exudate. The cellular component of the alveolar exudate varies in different animals, and in various parts of the same lung. The earliest and primary cellular response consists of a mobilization of numbers of large mononuclear phagocytes within the alveoli. Mitoses are numerous in these cells. A few polymorphonuclear leucocytes are occasionally present. In places where portions of the alveolar walls are necrotic, and in alveoli about bronchi with necrotic epithelium, polymorphonuclear leucocytes predominate. Cases that showed bacteria in the lung, either on culture or in stained sections, were discarded.

The most characteristic change is found about the blood vessels. This consists of an enormous dilatation of the perivascular lymphatics which often contain fibrin and many cells. The connective tissue of the adventitia is edematous and at times infiltrated with mononuclear and polymorphonuclear cells. In advanced cases the entire wall of large arteries is edematous and the muscle cells of the media pushed apart. Polymorphonuclear leucocytes often infiltrate all coats of such vessels, and in places collect in groups beneath the intima, lifting it away from the wall.

Both the large and small bronchi show localized areas of necrosis in the mucosa. Hordes of polymorphonuclear cells collect in and about the necrotic epithelium and extend through the bronchial wall. In the submucosa the connective tissue is edematous and the blood vessels dilated.

After extensive search cytoplasmic inclusions of the Guarnieri-body type were found in the bronchial epithelium. They are round or ovoid, have the charac-

teristic staining reactions, are surrounded by a clear zone in the cytoplasm and often indent the nucleus. These were found in only a few animals and then were present in only a few bronchi. Guarnieri bodies were never seen in the alveolar epithelial cell. A great variety of "inclusions" can be found in the degenerating bronchial and alveolar epithelium, some of which are misleading, but typical Guarnieri bodies possessing the characteristics mentioned, although scarce in our material, where found, can be confused with nothing else.

Immune animals show little or no reaction. Small collections of mononuclear cells in the alveoli and slight edema of the adventitia of blood vessels is usually found. Necrosis is minimal and polymorphonuclear leucocytes are scant. Guarnieri bodies were not found in immune animals.

Normal testicular extract was without effect, while heated virus, virus mixed with immune serum, and herpes virus produced slight reaction that was not to be confused with that of the active virus.

Discussion

(Dr. Ralph D. Lillie, Washington.) In the work that we did on vaccine virus pneumonia, judging from the pictures that Dr. Muckenfuss has shown, in our material we produced less cellular reaction in the lung, and more of the edema and fibrin type, with more necrosis; the perivascular edema was more marked in our material than in the photographs shown, and we also found bodies which we were inclined to interpret as inclusions, more widespread and more frequently. The virus used, however, was a different strain, and I believe that the differences are to be assigned to that difference in virus. From other work in the literature, I concluded that the virus used by Armstrong and myself produced more necrosis than that of Levaditi or than that used in this work.

(Dr. Max Pinner, Tucson.) Is anything known of the late results, and is there any marked amount of destruction of pulmonary tissue?

(Dr. Lillie.) Our own animals all died. The longest survival we had was eight days, and that showed almost total necrosis of lung tissue over widespread areas.

(Dr. Muckenfuss, closing.) We killed our animals in five to seven days; none were kept longer, and a number of the animals did die, but because of the extreme infiltration around the point of inoculation, we were never certain whether it was a death resulting from strangulation or from pneumonia. In some animals the lung destruction was not extensive.

A Comparative Study of the Histopathology of Experimental Tuberculosis in Different Species. William H. Feldman, Rochester, Minn.

Abstract. Using pure strains of *Mycobacterium tuberculosis* representative of the human, bovine and avian forms of the organism an experimental tuberculous infection was induced by various procedures in chickens, rabbits, guinea pigs and dogs. From the lesions obtained a comparative study was made to determine if the anatomical character of the cellular reaction in the respective species varied with the bacillary type of the bacteria used to incite the lesions, or if the histological character of the lesions in a given species was constant, regardless of the form of *Mycobacterium tuberculosis* present.

1. Variations in the character of the tissue reaction of *Mycobacterium tuberculosis* are not determined by the bacillary type of the organism *per se*, but by

the certain indefinite factors which are inherent to the species possessing the infection.

2. The histopathological nature of tuberculous infections in a given species is essentially the same, regardless of the form or origin of the particular strain of *Mycobacterium tuberculosis* responsible for the lesions.

3. The lesions of tuberculosis in the common fowl usually possess anatomical characters which differentiate them from tuberculous lesions in the lower mammals.

4. These observations would indicate that it would be injudicious to designate a given tuberculous infection as being due to organisms of either human, avian or bovine origin in the absence of the results of pathogenicity tests.

Discussion

(Dr. Esmond R. Long, Chicago.) I should like to ask Dr. Feldman if any correlation was apparent in these experiments between hypersensitiveness in the animals used, and the type of infection caused in them. The reason I ask the question is this: my associate, Dr. Vorwald, has made a somewhat similar study in which he inoculated with the same type of tubercle bacillus a whole series of animals, including the monkey, rat, guinea pig, chicken, dog, cat, rabbit, and turtle. A varying character of the resulting lesion was noted, which in some respects could be correlated with the allergic state. The more allergic the animal the more likely the tubercles are to be diffuse. In allergic animals the tubercle bacilli, as they spread, fall on sensitized soil. The guinea pig becomes profoundly sensitive to tubercle bacilli, and as the lesion spreads, there is a diffuse type of reaction. The rabbit becomes less sensitized and the tubercles are sharply delimited. The anatomical type of lesion is thus correlated with the variation in sensitiveness in the two animals.

(Dr. Feldman.) We have made no studies of that kind.

(Dr. David L. Belding, Boston.) May I ask if there were any observations made on the number of organisms present in the lesions? One of the characteristics of avian tuberculosis is the relatively large number of organisms present in the lesion.

(Dr. Feldman.) We made studies some time ago with the avian form of organism, injecting it into the brains of dogs, and while the organisms may be demonstrated, they were very few in number, but the same organism in chickens is very frequent.

(Dr. Oskar Klotz, Toronto.) Dr. Feldman has presented an interesting study of the comparative tissue reactions arising in different animals, resulting from infection with human, bovine and avian strains of tubercle bacilli. I am interested to know whether Dr. Feldman has had the opportunity of studying the effects of the avian infection in man. We have had the opportunity of examining the tissues of three cases of avian infection in man, in which the avian organisms were isolated and differentiated by Dr. R. M. Price. In two of these cases there was a generalized glandular enlargement, and they were diagnosed clinically as Hodgkin's disease. Histologically, however, the lesions were not those of Hodgkin's disease, but showed all the characteristics demonstrated by Dr. Feldman in the avian lesions in fowl. It is our belief that the individual tissue reactions are related to the quality of the organism and not solely the peculiarity of the host in which the reaction occurred.

(Dr. Feldman.) We have had no experience with cases diagnosed clinically as avian tuberculosis. We did some work with Hodgkin's disease, and we have one case which looks as though it might be associated with avian tuberculosis. There was no opportunity to study the lesion such as you mentioned.

(Dr. Stuart Mudd, Philadelphia.) Do all of these lesions progress to death, or do some of them regress? Can you fit into your interpretation tuberculosis of the Yersin type and that which occurs in the white rat, in which the organs are riddled with tubercle bacilli but there is little cellular response?

(Dr. Feldman.) I think it is a fair question to ask about the duration of the lesions. It is reasonable to assume that the character of the lesions might have changed with the duration of time, but in many of the animals, particularly the guinea pigs, the duration of the lesion, especially when the injection was with the human or bovine types, was short. In the chicken the lesions produced by the bovine type extended in one case for 136 days. The bird was perfectly normal in appearance, and when we destroyed it and made sections there were present very definite lesions of tuberculosis, limited, however, to the brain.

(Dr. David Perla, New York City.) It might be interesting in this regard to note that tuberculous infection in the white rat gives a different pathological picture from that in other animals. The tubercle in the white rat, produced by the human, bovine or avian type, consists largely of epithelioid elements. There is no accumulation of lymphocytes or any giant cells of the Langhans type, but just masses of epithelioid cells, and in these one sees tubercle bacilli. After a time giant cells form by fusion of epithelial elements. The nuclei of such giant cells are equally distributed throughout the cell.

(Dr. E. M. Medlar, Mt. McGregor, N. Y.) If you have tubercle bacilli of sufficient virulence in large enough numbers you may not get a tubercle at all. Instead the inflammatory response will go directly to the production of an abscess composed almost entirely of neutrophiles without the intervening stage of tubercle formation.

THE CELLULAR REACTION TO INFECTION WITH TUBERCLE BACILLI. EXPERIMENTS ON THE CORNEA IN ANIMALS OF VARYING SUSCEPTIBILITY. Esmond R. Long, and (by invitation) Arthur J. Vorwald and Sion Holley, Chicago, Ill.

Abstract. Previous studies by Vorwald have shown that in the lungs of animals of greatly varying susceptibility to tuberculosis the ultimate spread of the disease parallels more often the intensity of the initial response than the reverse; and that in the rabbit prompt phagocytosis of injected tubercle bacilli by polymorphonuclear leucocytes occurs, with subsequent phagocytosis of the latter by large mononuclear exudate cells. The present study was undertaken to study the rôle of the respective exudate cells in the less complex, non-vascular cornea in animals of greatly varying susceptibility. Human type tubercle bacilli in a dosage of approximately 0.001 mg. were injected, under light ether anesthesia, into the center of the cornea in cats and rabbits and normal and tuberculous guinea pigs, previous experience having shown that cats are highly resistant to the strain of bacillus used, while rabbits are less so and guinea pigs are very susceptible. Tuberculous guinea pigs were used, also, to determine the effect of sensitization on the cellular response. Animals of each group were killed after 6, 24 and 72 hours in order to study the character of the immediate reaction.

In all animals a cellular reaction at the limbus was noticeable 6 hours after injection. This was extensive and approximately equal in normal and tuberculous guinea pigs, much less marked in the rabbit and still less prominent in the cat. At 24 hours in all animals polymorphonuclear leucocytes had reached the site of bacillary lodgement in the center of the cornea, in enormous numbers in the tuberculous guinea pig, in less but still large numbers in the normal guinea pig, and in small numbers in the rabbit and cat. At 72 hours a massive abscess was present in the center of the cornea in the tuberculous guinea pig, while in the other animals the zone of inflammation was much more restricted. In all cases, however, it was sharply localized, and the overwhelming majority of cells present were polymorphonuclear leucocytes. In all cases these cells had phagocytosed and brought the bacilli together from a considerable spread of corneal tissue, thus effecting a rapid concentration in a small area.

The results thus confirm previous studies showing that the localization of tubercle bacilli in a restricted area after diffuse infection, but before the development of a true epithelioid tubercle, is brought about by polymorphonuclear leucocytes. The extent of cellular outpouring was a direct measure of the susceptibility of the animal, as far as the different species were concerned. Apparently the less effective the native bacillocidal power of an animal, the greater the mass of its leucocytes called into action at the outset of infection. The increased outpouring of polymorphonuclear leucocytes in the tuberculous guinea pig as compared with the normal, on the other hand, indicates that this is a sensitized cell in tuberculosis and the massive reaction in this case may possibly be looked upon as a manifestation of acquired immunity.

The results throw light on, but do not solve, the question of the origin of the epithelioid cell in tuberculosis. Inasmuch as primitive tissue mononuclear phagocytic cells seem to be few in the cornea, the absence of mononuclear phagocytes in the 72 hour tubercle in this organ, in contrast to their concentration at this time in tubercles in organs where such cells are normally abundant, might be taken as evidence that the epithelioid cell of the tubercle is of local tissue origin. On the other hand, mechanical conditions are more favorable for the influx of the active polymorphonuclear leucocyte in the dense collagenic cornea, than for the sluggish large mononuclears of the blood stream, and this fact may explain the predominance of the former in the tubercle produced in the cornea, and leave open the possibility of tubercle formation by large mononuclears of the blood stream in organs where their exudation and travel occur more readily.

THE EFFECT OF SECONDARY INFECTIONS ON EXPERIMENTAL TRACHOMA. P. K. Olitsky and (by invitation) R. E. Knutti and J. R. Tyler, New York City.

Abstract. A study was made of the rôle played by secondary infections in experimental granular conjunctivitis induced by inoculation of human trachomatous tissues or of cultures of *Bacterium granulosis*, and particularly in the reproduction of the florid type of trachoma in animals.

The implantation by a suitable technique of ordinary bacteria on the conjunctivae of monkeys already affected with well marked, characteristic *granulosis* lesions gave rise to a condition showing less predominance of the follicular reaction and more of the hyperemic granulopapillary effect, which resembled closely the florid type of human trachoma. The organisms used — diphtheroids, staphylococci, xerosis bacilli, chromogenic Gram-negative bacilli, spore-bearing Gram-negative bacilli, and non-hemolytic streptococci — were

some of those found in the conjunctiva of monkey and man with different types of granular conjunctivitis. By themselves, they produced only a transient reaction, or none; when inoculated together with material containing *Bacterium granulosis* they did not affect the usual action of this organism, but when introduced into a conjunctiva in which *granulosis* lesions were already well developed, they induced more of the characteristics of the florid stage of trachoma than have been hitherto observed in animals. Notably among the signs were increased hyperemia, edema, and thickening, papillary hypertrophy, obscurity of blood vessels and masking of the follicles. Microscopically these changes were accompanied by increased scar tissue formation, lymphoid and papillary hyperplasia.

THE INCIDENCE OF INTRANUCLEAR AND CYTOPLASMIC INCLUSIONS IN THE SALIVARY GLANDS AND OTHER ORGANS OF INFANTS. Sidney Farber, Boston, Mass.

Abstract. A study of the parotid and submaxillary glands of over 200 infants showed intranuclear and cytoplasmic inclusions in the ducts of more than 15 per cent of the cases. These inclusions are of the type originally described as "protozoan-like" structures, and more recently have been associated with certain types of filtrable virus disease. An analysis of the clinical and pathological data reveals no definite disease picture. One case showing these structures in various organs of the body is also described. Photomicrographs illustrating typical findings are presented.

Discussion

(Dr. Oskar Klotz, Toronto.) I would like to add a word about an interesting finding in a series of rats that was obtained by Dr. J. Thompson in the Nutrition Laboratory of the Hospital for Sick Children, Toronto. These rats, 2 months old, had been subjected to experiments on vitamin D deficiency over a short period. Tissues of the animals were examined histologically and in 70 per cent of them the submaxillary glands were studied. In 14 per cent of these, inclusion bodies were found in these glands which resembled very closely those described by Dr. Farber, as well as those occurring in the guinea pig. These inclusion bodies occupy the nuclei and almost completely obliterate them. There are some differences in size and staining characters between these inclusion bodies of the rat and those of the guinea pig. The animals show no evidence of disease, but microscopically the cells lining the ducts tend to desquamate and sometimes are associated with neighboring lymphoid infiltrations. In some previous work by Cole and Kuttner it was found that the guinea pig virus was not transmitted to rats and it would appear that the inclusion bodies found by Dr. Thompson are specific for this animal. Transmission experiments with the virus from the salivary glands of the rat have as yet not been undertaken.

(Dr. E. V. Cowdry, St. Louis.) I am very much interested in this presentation, because Dr. Wolbach very kindly sent me some of the specimens, and I compared them in detail with those in the guinea pig. I found that it was impossible to distinguish between the two inclusions, although the surrounding tissue is perhaps a little different. I am interested in the incidence of the inclusions, because Dr. Gordon H. Scott of St. Louis attempted to find them in newborn infants and fetuses collected in St. Louis, Minneapolis, and the middle west. He was not successful. In 100 individuals examined no inclusions were

found, which merely means that this virus is behaving like other viruses. In Boston, Dr. Farber has demonstrated a high incidence, while in the middle west it has not yet been reported, just as the virus causing inclusions in the guinea pig reveals itself by inclusion formation very frequently in some districts of the United States, and not at all in others.

THE PATHOLOGY AND BACTERIOLOGY OF BRONCHIECTASIS. A STUDY OF EIGHT SURGICAL LOBECTOMIES. W. L. Robinson and (by invitation) P. H. Greey, Toronto, Ont.

Abstract. This represents a study of eight cases of bronchiectasis in which a lobe or portion thereof was removed at operation and sent immediately to the laboratory for culture and sections.

Clinically, most of these represent cases in their developmental stage and therefore offered exceptional opportunity for a study of the incipient stages and the determination of possible etiological factors. Direct smears, dark field preparations, aerobic and anaerobic cultures of various types, as well as bacterial stains and silver preparations of sections, revealed nothing consistent enough to be pointed to as a specific infective agent.

Cultures were taken from the exudate in the lumen and also from scrapings of the wall. No common flora were recovered. Three cases yielded pure cultures of streptococci of hemolytic, non-hemolytic and viridans types respectively. Two showed mixed aerobic cultures. Two others gave a mixture of hemolytic streptococci, spirochetes, and fusiform bacilli. One other case gave a mixture of pneumococcus Type IV, with spirochetes and fusiform bacilli. In only three of the eight cases, therefore, were spirochetes found.

Histological sections of the bronchi, transverse and longitudinal, showed consistently the same type of chronic inflammatory reaction, namely a lymphocytic and plasma celled infiltration of the whole bronchial wall, most marked in the mucosa. Destruction of the bronchial musculature and elastic sheaths stood out prominently as the dominant factor in the weakening of the wall, leading to the bronchiectatic dilatations. These in some cases were shown to be focal in character, leading to sacular dilatations as well as to the fusiform or cylindrical types usually found. The lining epithelium as a rule consisted of three or four layers of columnar epithelial cells, the surface layer in most cases being ciliated. Under dark field illumination scrapings of these cells showed the cilia whipping for some six or seven hours after removal from the bronchus. In the more acute areas the lining epithelium was quite hyperplastic and irregular in arrangement, and showed occasional small ulcers or erosions with an acute inflammatory reaction in their bases. An endarteritis of the bronchial arteries was also found.

FOLLICULAR LYMPHOBLASTOMA (GIANT FOLLICULAR HYPERPLASIA OF THE LYMPH NODES AND SPLEEN). George Baehr, Paul Klemperer and (by invitation) Nathan Rosenthal, New York City.

Abstract. Further studies have been made of pathological material obtained from cases reported by Brill, Rosenthal and Baehr as giant follicular hyperplasia of the lymph nodes and spleen in 1925, and by Baehr and Rosenthal before the Association of American Pathologists and Bacteriologists in 1927 as malignant lymph follicle hyperplasia of spleen and lymph nodes. In lymph nodes or spleen obtained at operation early in the disease, enormously enlarged lymphoid follicles characterize the microscopic picture. They resemble huge germinal centers consisting of lymphoblasts, in which mitotic figures are frequently seen.

The periphery of each large follicle is surrounded by a narrow zone of small lymphocytes with darker staining nuclei. No abnormal cells are found in the blood.

At this stage of the process it can be differentiated from simple follicular hyperplasia of inflammatory or constitutional nature by the frequency of mitotic figures, the greater size of the follicles, the tendency to involvement of the fibrous capsule, and, when the spleen is involved, by the splenomegaly which may reach the weight of 1800 grams. The differential diagnosis from a focal infiltration of the node by Hodgkin's lymphogranuloma can usually be made by the different architecture of the node. The gross appearance of the lymph node is of value, for the huge follicles are often macroscopically visible. The gross appearance of the spleen is also quite different from the porphyry appearance of Hodgkin's disease. In its early phase the condition is pathologically related to the systemic hyperplasias of the lymphatic tissue, although it differs from the common histological picture of an aleucemic lymphadenosis because of the striking follicle formation, and the predominance of lymphoblasts.

In material obtained later in the disease from the same individuals and from four autopsies, the pathological process was seen to have undergone a change. The growth of the pathological follicles resulted in conglomerate fusion so that the characteristic picture of lymphosarcoma had resulted. At this stage, no other diagnosis than lymphosarcoma is possible, the lymph nodes being diffusely infiltrated by polymorphous lymphoblasts with large irregular nuclei, often showing mitoses. The capsule of the lymph node is infiltrated and there is diffuse invasion of the adjacent tissue. But even at this late stage a careful survey of the architecture of the neoplasm still reveals remnants of the original structure. Narrow bands of small dark staining lymphocytes which originally encircled the follicles can still be made out here and there, and with care one can still distinguish the outlines of some of the overgrown follicles.

In four cases the previous splenomegaly was not present at autopsy, in one a splenectomy having been performed, and in the other three cases the spleen having returned to normal size after roentgen therapy. In the latter, the spleen of one, though of normal size, showed gross nodular infiltration and in all these microscopic examination revealed several enlarged follicles presenting the characteristic change. The disease is therefore a form of lymphosarcoma which deserves to be distinguished as a pathological entity because of its characteristic pathology, its unique pathogenetic evolution and its unusual duration. It may form a connecting link between the systemic hyperplasia of the lymphatic tissue and lymphosarcomatosis, the possible transformation of a "pseudoleucemia" into lymphosarcomatosis having been already mentioned by Kundrat. It is clinically distinguishable from the common type of lymphosarcomatosis by its chronicity (one case being still alive after twelve years), the tendency to early general lymphadenopathy and splenomegaly, the complete absence of anemia or cachexia until the end stages of the disease, and the tendency to lymphatic infiltration in the lachrymal gland resulting in unilateral exophthalmos.

Its most remarkable distinguishing characteristic is its radiosensitivity. According to the experience of our roentgen department, there is no neoplastic disease which responds as promptly to relatively small doses of X-ray or radium therapy. Recurrences in distant parts of the body usually occur after varying intervals until eventually, often after many years, they become less easily influenced by radiotherapy.

In order to distinguish it from other varieties of lymphosarcoma, the designation "follicular lymphoblastoma" is proposed.

The microscopic pictures shown in the lantern slides illustrate the gradual and progressive change in the pathological process over a period of years from the appearance of giant follicular hyperplasia to a characteristic infiltrating lymphosarcoma.

Discussion

(Dr. Howard T. Karsner, Cleveland.) I think we have all been very much enlightened by being brought up to date on the question of giant lymph follicle hyperplasia. What impresses me about this presentation, as well as some few personal observations I have made, is the doubt which may arise about the justification of thinking of this lesion as confined to the lymph follicles. Dr. Klemperer, for instance, refers to the increase in the number of follicles in the spleen. I think he would also say, if he used the same terminology, that there is an increase of the follicles in the lymph nodes. Are we justified in referring to the many centra as indication of an increase in the follicles, or as a number of new foci of the disease that are not in themselves primarily lymphfollicular?

(Major George R. Callender, Washington.) One of Dr. Symmer's cases, which was loaned by the Army Medical Museum, terminated with an even more malignant picture than is shown in the slides here. The case was reported in full at the Chicago meeting in 1929, from the Lymphatic Tumor Registry.

On So-Called Hematogenous Peritonitis. Otto Saphir and (by invitation) S. Wile, Chicago, Ill.

Abstract. This study is based on seventeen cases of peritonitis in children. The peritonitis was diagnosed clinically as hematogenous in origin. Nine cases were operated upon and the diagnosis apparently confirmed because no primary source of the peritonitis could be detected within the peritoneal cavity. The autopsy revealed in all the cases a primary enteritis and secondary peritonitis. Some of the changes in the intestines were only slight grossly, but histologically were found to be much more advanced. An oxydase stain revealed the presence of polymorphonuclear leucocytes within the muscular coat where they could not be made out by the use of the hematoxylin-eosin stain. A Gram-Weigert stain showed bacteria in the muscularis in some of the cases. In six cases, hemolytic streptococci were cultured from the peritoneal exudate, in two cases pneumococci, and in two cases, *B. dysenteriae* (Shiga), and *B. typhosus* respectively. Cultures taken from the intestinal mucosa revealed hemolytic streptococci in three of these cases, pneumococci in two, and *B. dysenteriae* and *B. typhosus* in two respectively. The peritonitis in these cases is explained by an invasion of the peritoneum by bacteria through the intestinal wall. This type of peritonitis is referred to as *Durchwanderungs-Peritonitis* in the German literature (emigration or migratory peritonitis).

In reviewing the literature on hematogenous peritonitis, difficulties were encountered because many cases are reported without autopsies, or with autopsy but without detailed investigation of the gastro-intestinal tract so as to rule out a migratory peritonitis. The common belief is that a primary upper respiratory infection gives rise to a bacteremia. The bacteremia secondarily produces the peritonitis. There is no explanation why the peritoneum alone should be involved, and no other organ, especially not the bone marrow, which is a favored

site of localization in bacteremia in childhood. Furthermore, it is not at all clear how bacteria reach the peritoneal cavity from the blood stream in the absence of an intermediary lesion. It is conceivable that hematogenous peritonitis might occur, but only with intermediary lesions, such as abscesses in the mesenteric attachment or in organs within the peritoneal cavity, or infected thrombi. The peritonitis in these cases, however, is the result of such metastatic pyemic abscesses or thrombi, but does not directly follow the blood stream infection. So-called hematogenous peritonitis, however, is a peritonitis which supposedly directly follows a blood stream infection. There is no proof that such a peritonitis does exist without other metastatic pyemic phenomena. It seems that many cases reported as hematogenous peritonitis are examples of migratory peritonitis (*Durchwanderungs-Peritonitis*).

Discussion

(Dr. Henry J. Gerstenberger, by invitation, Cleveland.) A case such as Dr. Saphir described was seen in the Babies' and Children's Hospital. It was an infant 8 weeks old who was in extreme collapse and presented sclerema. A peritoneal puncture revealed a cloudy fluid and a lumbar puncture also presented pathology, as the fluid contained an increased number of cells. I would like to ask Dr. Saphir if in any of the cases reported by him the lumbar fluid was examined.

(Dr. Saphir.) There was no evidence in any of our cases of involvement of the meninges, and a lumbar puncture therefore was not done.

(Dr. Gerstenberger.) I might explain why we did a lumbar puncture in this case. Oftentimes in these infants the general picture does not seem to be that of a peritonitis, but rather that of an encephalitis. As a matter of fact, the first patient with peritonitis that I had occasion to see was diagnosed by me as encephalitis because of the absence of peritoneal signs and the presence of severe cerebral collapse. The lumbar fluid, however, in this first case was negative, and the autopsy revealed the presence of a peritonitis.

(Dr. E. T. Bell, Minneapolis.) I should like to ask if these changes in the wall of the intestine might not be interpreted as secondary to the peritonitis and not as primary. In any case of advanced primary peritonitis you see changes in the muscle of the intestine. It would be curious if a mild enteritis should cause a peritonitis when we know that a frank outspoken enteritis does not cause peritonitis, except in perforation.

(Dr. Saphir.) We have control sections taken in cases of perforation of the intestine. In those sections which were taken from other portions than from the site of perforation, and which were stained for oxidase granules, we could not find any polymorphonuclear leucocytes in the muscularis. In many of the cases the enteritis was grossly much less marked than might have been expected from the histological picture. It does not seem possible to me that a primary peritonitis should produce a secondary enteritis extending over long stretches of the intestine—for instance, over the entire gastro-intestinal tract. It does not seem to me that the enteritis in such cases could be secondary to the peritonitis.

(Dr. David P. Seecof, Cleveland.) I should like to ask if the bone marrow from these cases was examined culturally.

(Dr. Saphir.) The bone marrow was examined microscopically and grossly in some cases, but nothing was found. In a few instances the bone marrow was cultured, and nothing found. In two or three cases, the cultures of the bone

marrow were positive, but we explained that as secondary to the bacteremia, and the bacteremia secondary to the peritonitis.

(Dr. I. Davidsohn, Chicago.) Was there any relation between the type of organism and the sex? In a number of cases which I had the opportunity to observe we almost exclusively found pneumococci in girls, and streptococci in boys. Did you notice any infection of the genital tract? In some of the cases which I saw, there was evidence of vaginitis in the girls, as a primary infection.

(Dr. Saphir.) Two cases showed pneumococci in the peritoneal cavity; one was a girl and the other a boy. The vagina, uterus, ovaries and tubes were carefully examined, but we found no evidence of inflammation in any of the sections.

(Major George R. Callender, Washington.) Were the pneumococci typed?

(Dr. Saphir.) Yes, they belonged to pneumococci Type II in one case, and Type IV in the other.

(Dr. Norbert Enzer, Milwaukee.) Was there any evidence of pulmonary infection?

(Dr. Saphir.) Two cases showed bronchopneumonia.

(Dr. Shields Warren, Boston.) During this upper respiratory infection was there a positive blood culture?

(Dr. Saphir, closing). The blood cultures were done after the patients were admitted to the hospital and clinical signs of peritonitis had developed. Therefore no blood cultures were taken early in the disease when the upper respiratory infection occurred.

TEETH AND EPIPHYSES IN DEFICIENCY DISEASES. S. B. Wolbach, Boston, Mass. Presented by Shields Warren, Boston.

Abstract. The consequences of a rachitogenic diet and diets deficient in vitamins A and C upon growth of incisor teeth and long bones of rats and guinea pigs are compared and contrasted.

In teeth a rachitogenic diet affects calcification; a C deficient diet causes cessation of formation of the matrix of dentine, and an A deficient diet operates through atrophy of the enamel-forming epithelium and, through atrophy and heteroplastic proliferation of odontoblasts, produces the most profound changes in the teeth of any of the three deficiencies.

In bones a rachitogenic diet affects calcification of cartilage and bone matrices and suspends the sequences of cartilage cell changes preliminary to vascular ingrowth; the proliferative rate of the epiphyseal cartilage is accelerated. The C deficient diet suspends formation of connective tissue, bone and cartilage matrices, resulting in complete solution of continuity at the epidiaphysial region. An A deficient diet suspends growth and brings about conditions approximating those of the adult epidiaphysial region.

Emphasis is laid upon the importance of the repair phenomena in each instance for the understanding of the pathogenesis of the various disturbances discussed. In repair of the rachitic and scorbutic deficiencies one sees normal sequences taking place at an accelerated rate, and the character of the repair here is largely that affecting intercellular substances. In A deficiency repair depends on the restoration of cells to normal states and hence there is a considerable apparent lag in response to restoration of diet.

EARLY LESIONS OF RHEUMATIC ENDOCARDITIS. Timothy Leary, Boston, Mass.

Abstract. The material presented consists particularly of early lesions of endocarditis obtained from three cases: one dying as the result of violence while in

apparent full health (Case I); one dying on the way to the hospital (Case II), and one dying of organic disease unrelated to the cardiac process (Case III).

These cases present a valvular lesion marked by the formation of a palisade of cells lining the contact edges of the valves. The early stage of this lesion has not been described. In Case I (early phase) the palisade was covered by a thin layer of fibrinous exudate throughout which Gram-positive diplococci were distributed singly or in small masses. In the necrotic valve edge at one point a larger mass of bacteria was found either lying in a vessel or in a crevice in the valve surface. There were no vessels in its neighborhood. There was no acute inflammatory process in the valve tissues. Cultures developed a pure growth of *Streptococcus viridans*. There were no verrucae on the valve.

Case II (intermediate phase) showed an old mitral stenosis. On the tricuspid valve occurred crops of verrucae and a yellow-brown layer traversing the whole contact edge. The yellow-brown layer consisted of a palisade covering the interverrucal region and the surfaces of the verrucae. All stages in the evolution of the verruca from early budding processes to advanced stages were present. In addition diffuse formation of scar tissue direct from the palisade cells could be demonstrated. No bacteria were found.

In Case III (later phase) an old degenerating stage of the palisade persisted on a chronic mitral valve on which pseudoverrucae had formed. These were made up of clots deposited on the necrotic scarred valve edge. This stage has been described and illustrated by Königer. Remains of the palisade were observed on rheumatic valves from other cases in the verrucal stage, in which the process was not too advanced.

The lesion represents, apparently, a reaction of allergic tissue to injury. The presence of diplococci on the surface of the valve showing the earliest, most perfect process, is suggestive.

As Poynton has indicated, our difficulties with reference to etiology and diagnosis in rheumatism are "increased by the great rarity of fatal rheumatic heart disease in the very earliest phases of the illness in man." The lesions described in this paper, it is believed, represent an early process whose character helps to elucidate the evolution of the classical picture of verrucose rheumatic endocarditis.

Discussion

(Dr. Benjamin J. Clawson, Minneapolis.) I should like to ask Dr. Leary if in the course of his coroner's work he has seen an aortic lesion with extreme calcification, the so-called arteriosclerotic valve, and what is his interpretation concerning the etiology and pathogenesis of this aortic lesion?

(Dr. Leary.) I have already discussed this question with Dr. Clawson, and we are in agreement. It is my opinion that this calcified lesion of the aortic valve referred to as arteriosclerotic is basically of rheumatic origin. I think the end result is calcification. I know that this is not in general agreement with the world at large, but there are a good many things on which Dr. Clawson and I agree, and which do not agree with the world at large.

(Dr. Norbert Enzer, Milwaukee.) I should like to ask Dr. Leary if he stained organisms in the valve as well as on the surface of the valve.

(Dr. Leary.) I did not make it plain that the exudate must have arisen wholly from the blood flowing over the surface of the valve. There were no bacteria in the valve other than this mass which I described. We could not find any vessels, and the valve was not vascularized, except at the base.

(Dr. E. C. Rosenow, Rochester, Minn.) The lesions in the valve shown by Dr. Leary remind me very strongly of the lesions in the valves which I found in rabbits following the intravenous injection of *Streptococcus viridans*. Ulceration and vegetation formation are usually found first along the line of closure, even though the initial lesion is subendothelial, and in this beautiful example, where the bacteria are numerous, and where there is ulceration, why could not these masses in the depth of the valve be the cause of the thin fibrinous deposit on the surface, which so strikingly seems to be from the circulation? In the experiments in rabbits it was demonstrated that ulceration along the line of closure, suggesting implantation endocarditis, was nevertheless secondary to antecedent embolic subendothelial hemorrhage and growth of the streptococcus. This is obviously difficult to demonstrate in the human, but readily accomplished in experimental animals by sacrificing them at intervals following injection. Capillaries surrounding the embolic lesion were not always demonstrable.

(Dr. Leary, closing.) I had in mind, of course, Dr. Rosenow's splendid demonstration of the production of embolic endocarditis when I discussed this case. We could not find any vessels. There are two possibilities: the bacteria were deposited on the surface from the overflowing blood, or they were delivered embolically. No matter how they got there, the action of the closing and opening of the valve is a mechanism adapted for the distribution of the organisms over the contact edge of the valve. We could not find any vessels on careful search which might cause an embolic lesion at the point where the bacterial mass was found.

GLOMERULAR LESIONS IN ENDOCARDITIS. E. T. Bell, Minneapolis, Minn.

Abstract. The embolic lesions described by Löhlein were for a long time considered the only glomerular alteration in bacterial endocarditis. Baehr and Lande, however, called attention to the frequent occurrence of acute diffuse glomerulonephritis in association with bacterial endocarditis. Clawson and Bell corroborated this observation. In this study I have found that about two-thirds of all cases of bacterial endocarditis and about one-third of all cases of acute rheumatic endocarditis show a definite acute diffuse glomerulonephritis at postmortem.

In preparations stained with hematoxylin-eosin the glomeruli show a definite increase of nuclei, but they are smaller and the capillary obstruction is much less than in clinical acute glomerulonephritis.

Preparations stained by the Mallory-Heidenhain method (McGregor) show a striking increase in the number and size of the capillary endothelial cells. It differs, however, from clinical acute glomerulonephritis in the absence of intracapillary fibers and in the fact that the capillary obstruction is not so pronounced.

A similar increase of glomerular endothelium is frequently seen in persons dying of septic processes such as lobar pneumonia and streptococcic bacteremia.

The presence of large numbers of bacteria in the circulating blood often causes glomerulitis.

Discussion

(Dr. Paul Klemperer, New York City.) I should like to ask Dr. Bell in how many cases of subacute bacterial endocarditis he found actual glomerulonephritis of the clinical type. These lesions which Dr. Bell has demonstrated are very often found by us in subacute bacterial endocarditis, I think in about the

same percentage as Dr. Bell has mentioned. They were especially impressive in cases in which the influenza bacillus or the gonococcus was the cause of the subacute bacterial endocarditis. In addition, however, we find in a certain number of cases actual glomerulonephritis. Furthermore, a type of renal lesion occurs where there is also clinical evidence of renal damage with death in uremia, in which so great a number of glomeruli are destroyed by the focal emboli glomerulitis that the kidney becomes insufficient.

(Dr. Bell, closing.) There are a few of these cases, I do not recall the actual number, in which there was a definite clinical acute glomerulonephritis, but it is difficult to decide that. If there is edema present, it might be due to the decompensated heart, but in a considerable number in which the blood chemistry has been studied, there is a marked nitrogen retention, so that perhaps some of these may be put in the group showing renal insufficiency. I do not think this lesion is due to any specific organism. It occurs with pneumococci, both kinds of streptococci, hemolytic and non-hemolytic, and rarely with staphylococci.

AN INTERPRETATION OF THE NATURE OF HODGKIN'S DISEASE. E. M. Medlar, Mount McGregor, N. Y.

Abstract. The material made available for this study consisted of tissues from 22 autopsies of Hodgkin's disease and about 100 surgical specimens diagnosed as lymphoblastoma, lymphosarcoma, and Hodgkin's disease.

Evidence was found which made it seem probable that Hodgkin's disease is a dyscrasia of the hematopoietic tissue of the bone marrow. It appears that the "stem" or parent cell of the hematopoietic tissue of the marrow is a multipotential cell which gives rise to all of the cell types of the blood known to be produced in this tissue. This leads one to believe that Hodgkin's disease is closely related, genetically, to the myelogenous leukemias and to the erythroblastic dyscrasias. The essential difference between these dyscrasias is that in neutrophilic myelogenous leukemia the parent cell differentiates mainly along the line of the neutrophile, while in Hodgkin's disease the differentiation is chiefly along the line of the megakaryocyte. The complex pathological manifestations of marrow hematopoiesis, such as seen in pernicious anemia, myelogenous leukemia, and Hodgkin's disease would, from the evidence found, revert to a single type of cell which is multipotential. To emphasize that all of these pathological processes are concerned, genetically, with but a single type of cell, the author suggests that such a term as myelogenous leukemia or Hodgkin's disease should be used, merely to indicate that the main line of differentiation of the parent cell is toward the neutrophile or eosinophile or the megakaryocyte.

The author is of the opinion that Hodgkin's disease is a neoplastic manifestation of bone marrow hematopoiesis and that the involvement of tissues outside of the marrow may be regarded as metastatic tumor growths. To indicate that the multipotential parent cell differentiates mainly along the line of the megakaryocyte and blood-platelet in Hodgkin's disease, the term megakaryoblastoma is suggested.

Discussion

(Dr. Samuel R. Haythorn, Pittsburgh.) I have been very much interested in Hodgkin's disease from the standpoint of the cytology, though I am by no means sure of the sources of the so-called "Dorothy Reed" cells. We have been working with a strain of *Monilia* which produces abscesses. In the walls of these

abscesses are two types of large mononuclear cells. One type is quite definitely the cell called the mononuclear leucocyte, or endothelial leucocyte. The cells of this strain are phagocytes and fuse to form foreign body giant cells. The other kind resembles the type of cell which Dr. Medlar has just described so closely that I am unable to differentiate them. They respond to the monilial reaction as inflammatory cells and then pass to nearby lymph nodes and may occasionally be seen there in mitosis. I have not been able to reproduce the picture of Hodgkin's disease in spite of the fact that typical Hodgkin's cells have occurred in the lesions. It seems to me necessary to assume a tumor-like character to these exudative cells to complete the lesion of Hodgkin's disease. For example, if we assume that certain wandering cells in persons susceptible to Hodgkin's disease may possess tumor qualities, may be capable of responding as inflammatory cells to any one of several bacterial stimulants, and may then pass to the regional lymph nodes and take on accelerated neoplastic growth, we have a theoretical explanation of the disease which coincides with the pathological findings. If no tumor quality is necessary, it seems that some of the irritants which call out the type cells in animals should occasionally produce the picture of Hodgkin's disease, and so far that has not been possible.

(Dr. J. P. Simonds, Chicago.) I should like to ask Dr. Medlar if he has any information in regard to the number of blood platelets present in the circulating blood in Hodgkin's disease. Some work by Dr. F. D. Gunn in our laboratory shows a definite correlation between the number of platelets in the circulating blood and the number of megakaryocytes in the bone marrow. He was fortunate enough to isolate from a subcutaneous abscess in a rabbit a bacillus which has a specific action, in that it stimulates the production of blood platelets up to, in one or two instances, three and a half million per cubic millimeter of blood, and there was a corresponding increase in the number of megakaryocytes in the bone marrow. If the giant cells in Hodgkin's disease are megakaryocytes, it would be interesting to know whether they contributed blood platelets to the circulating blood, just as those in the bone marrow do.

(Dr. Medlar, closing.) Bunting has shown, years ago, that there is a marked increase in the blood platelets, and Minot has found immature megakaryocytes in the circulating blood in Hodgkin's disease. An increase in blood platelets is not specific for any one thing. They are increased in the circulation in many acute inflammatory processes. An increase in blood platelets is often observed in acute tuberculosis. Megakarocytes are occasionally found in large numbers in the lung in acute lobar pneumonia. It appears to me that the megakaryocyte and the blood platelet play an important part in early acute inflammatory reactions.

UNATTACHED TUMORS FROM THE EMBRYONAL UROGENITAL APPARATUS. G. H. Hansmann and (by invitation) J. W. Budd, Iowa City, Iowa.

(*Abstract not submitted.*)

ON THE PRODUCTION OF DECIDUAL CELLS IN AN OVARY ABOUT A PRIMITIVE CHORIO-EPITHELIOMA OF THAT ORGAN. Charles Simard (by invitation), Montreal, Canada.

Abstract. A primitive chorio-epithelioma of the ovary occurred in a woman aged 42, whose last confinement occurred six years ago. Since that time there was no evidence of normal or ectopic pregnancy.

The tumor is formed by strands which recall the chorionic villi of the placenta. They are bordered by large, flattened syncytium masses. Beneath the syncytial layer are cells that are similar to Langhans cells.

The tumor is a typical chorio-epithelioma.

In the ovarian tissue, but outside the invaded zones, are found sheaths of multistratified cells around capillaries or small vessels. Those elements are characteristic decidual cells.

In the case reported, where there are neither follicles nor corpus luteum, we have to deal with a tumor of pure chorioplacental elements, therefore with elements which represent the sole fetal placenta. Then it is not illogical to believe that the appearance of decidual cells is caused by a secretion of the chorio-epithelioma elements.

If the perivascular decidual cells of the tumor have been differentiated under the hormonal action of the chorio-epithelioma, it seems that the hormone which gives rise to the appearance and multiplication of the decidual cells, in normal pregnancy, may come exclusively from chorioplacental elements.

In other words, if the interpretation is correct, it suggests the existence of a hormone which, secreted by the elements of the fetal placenta, determines the production of the characteristic cells of the maternal placenta.

GRADING OF CARCINOMA OF THE CERVIX UTERI AS CHECKED AT AUTOPSY. Shields Warren, Boston, Mass.

Abstract. While the grading of malignancy is not uniformly applicable to the individual case, it is not infrequently of value, particularly from the standpoint of determining radiosensitivity. The duration of life alone does not give a fair criterion of the malignancy of a given tumor, as many persons do not die of the tumor itself, but of other conditions, which may or may not be related. While perhaps one-sided, the power of metastasis is a useful objective criterion, particularly when the extent of metastases is determined postmortem.

When a given case is followed through to death, variations in the clinical factors do not have to be so heavily weighed; variations in type and efficacy of treatment are not so important; and the grading of the tumor can be based on the entire specimen rather than on a biopsy, which may or may not accurately represent the tumor as a whole.

This study is based on seventy-six autopsied cases of carcinoma of the cervix. A modification of Broder's classification was employed, recognizing the three grades of epidermoid carcinoma, the adeno-acanthoma, and the adenocarcinoma.

In two of the fatal cases no tumor was found at autopsy. Of the twenty-one cases of Grade I epidermoid carcinoma, 86 per cent either did not metastasize, or their metastases were restricted to the regional lymph nodes. Of thirty-four Grade II epidermoid carcinomas, 44 per cent were so restricted. All of the four adeno-acanthomas had spread beyond the regional lymph nodes. Twenty-five per cent of the four adenocarcinomas were restricted to the regional lymph nodes. Thirty-six per cent of all cases examined showed visceral metastases. The commonest single cause of death was obstruction of urinary outflow.

PATHOLOGY OF ONE THOUSAND CERVICES. James E. Davis, Detroit, Mich.

Abstract. Consecutive examination of a large group of cervices stimulates new evaluations.

Infections coincident with or consecutive to lacerations and in association with erosions, ectropion, retention cysts and continued irritation constitute frequent composite lesions of importance second only to neoplastic changes.

Precancerous criteria may be interpreted only as possible indications of ultimate cancer development.

The grading of malignant growths should be influenced by clinical data.

Discussion

(Dr. Alfred Plaut, New York City.) I was very much interested in hearing about forty benign tumors of the uterine cervix in 1000 cases. I would like to hear what kind of tumors they were. In a large number of cervices I have observed, for five and a half years, I have seen a much smaller number of benign tumors.

(Dr. Davis.) There were six myofibromas, one endothelioma, and thirty-seven polyps.

(Dr. Plaut.) I never classify polyps as tumors.

(Dr. James Ewing, New York City.) I should like to ask Dr. Davis if he has any evidence that these carcinomas begin from what he calls the repair epithelium, or from the original epithelium.

(Dr. Davis, closing.) I was greatly impressed in this study by the changes taking place in the repair epithelium, in the presence of long continued inflammatory reaction. The picture of active repair, epithelial proliferation, nuclear polymorphism, and stromal scarring was repeated so many times that it became very impressive in sections definitely malignant and in those without malignancy. I think in a few sections where the repair epithelium was of second degree, that is, the first repair epithelium had been denuded and the second repair epithelium had appeared, that type of epithelium appeared peculiarly sensitive to continued infection, showing an appearance of unstable basal cells.

REPORT OF A SERIES OF ADRENAL ADENOMAS. Charles F. Branch, Boston, Mass.

Abstract. A series of seven cases of adrenal adenoma occurring within one year in a series of approximately 300 autopsies is reported. In three cases the tumors were bilateral, the largest measuring 2.5 cm. in diameter, the smallest 1.2 cm. in diameter. All were perfectly encapsulated, discrete masses. Microscopically they consisted uniformly of irregularly arranged groups of cortical cells, having a high fat content. It is interesting to note the complete absence of any clinical significance of the tumors. No two of the cases died from the same cause. The individual ages varied from two in their eighties to the youngest, 41, who died of a recurrent acute rheumatic fever. Four were in females, three in males. On careful examination no endocrine disturbance of any note could be found in even a single case.

Discussion

(Dr. David P. Seecof, Cleveland.) I should like to emphasize, as Dr. Branch pointed out, the association of these so-called adenomas with age. I do not know the exact figures, but in the autopsy series at the Montefiore Hospital in New York it would be safe to estimate that in people over 50 or 60 years of age such adenomas occurred in the adrenal in over 40 per cent of the cases.

The other point is the so-called adenomatous nature of these. I have come to regard the growths in the adrenals just as we regard the adenomas in the

thyroid and prostate. They are not really adenomatous, but localized areas of hyperplasia within misplaced adrenal rests. They occur only at puberty or the prepuberty periods. We would not expect signs of virilism in people 50 or 60 years of age.

FURTHER STUDIES OF INTRACRANIAL TUMORS BY SUPRAVITAL TECHNIQUE. Louise Eisenhardt (by invitation), Boston, Mass.

Abstract. An additional series of illustrations selected from the last hundred intracranial tumors which have been studied by this method, upon which the surgical clinic has come to depend more and more for immediate diagnosis, is presented.

Discussion

(Dr. Max Levine, New York City.) I should like to ask Dr. Eisenhardt if she has compared the supravital preparations with preparations made after fixing with some of the better cytological fixatives, and how long these supravital preparations last? They serve a purpose, but do they last?

(Dr. Eisenhardt.) They have not very lasting qualities. We use the method mainly for the purpose of immediate diagnosis, in order that the surgeon may know what kind of tumor he is dealing with, and what operative method to pursue. As fixatives we use formalin and Zenker solutions, except when special stains are to be prepared.

(Dr. Marcus W. Lyon, Jr., South Bend.) About forty years ago, when I was a student in zoölogy, we used to be able to fix hydroids with all the tentacles extended by pouring hot bichloride solution on them. It seems to me it ought to be possible to fix tissues so that they would retain their natural form just as easily as hydroids and barnacles. I think the trouble is in using poor fixation methods.

(Dr. Eisenhardt.) One of the purposes of using unfixed tissue is to study the cells while they are alive. We keep the preparations in a warm box, and observe the activity of the cells. We have permanent slides made at the same time of all the specimens examined.

(Dr. James Ewing, New York City.) I think the communication of Dr. Eisenhardt is of considerable importance. It shows it is not necessary to wait for the disintegration produced by the ordinary fixing and staining methods in order to get a diagnosis. For a quick diagnosis, these sections seem even superior to frozen sections. At the Memorial Hospital we have very largely abandoned frozen sections, and instead of that method we employ aspiration, putting in an 18 bore needle. One can get from almost any tumor sufficient fragments of tumor which are fixed in the air and stained with hematoxylin and eosin, and have a diagnosis in two or three minutes. That does not give you the advantage of the study of the living cell which Dr. Eisenhardt is particularly interested in, but it gives a diagnosis very quickly, and the cell forms are much better preserved than by other methods.

(Dr. William Boyd, Winnipeg.) After listening to Dr. Eisenhardt last year and looking with great admiration at her pictures, I tried out her method with fresh animal tissue. The result was a complete failure, and I was unable to get any satisfactory differentiation of cells. I should like to know, first, what kind of picture normal fresh tissue gives, and secondly, whether there are any special technical pitfalls into which I may have fallen. I wanted to try the method on normal tissue before using it on tumors.

(Dr. Eisenhardt.) In order to have good photographs for demonstration, I spend considerable time in finding the best area possible in the supravital preparations, but I spend an equal amount of time on the fixed preparations of the same tumor in order to select the best areas for comparison. Perhaps you use too much pressure on the material, which tends to destroy the cells. In a pituitary adenoma, for example, the cytoplasm is so fragile that only very gentle pressure should be used. The tissue ought to be examined very promptly also. Possibly your neutral red dye is not satisfactory.

(Dr. Boyd.) Should normal tissue give you the same staining reaction as the tumor does?

(Dr. Eisenhardt.) Yes. Possibly you will recall how clearly the muscle fiber invaded by tumor was seen in the supravital preparation.

(Dr. Esmond R. Long, Chicago.) I should like to mention a simple method, very crude in comparison with the one described, but of some use for quick work and where there are many sections to examine. It is to take a fresh surface and to touch it lightly to a slide, thus making a stamped impression of the tissue. A good many of the cells come off in their original interrelationship. You can see the original parenchymatous cells and the interstitial cells of inflammation, for example, and thus a rough impression is given of what is present in the tissue.

THE DIFFERENT TYPES OF CIRRHOSIS OF THE LIVER. F. B. Mallory, Boston, Mass.

Abstract. About twelve different types of lesions of the liver may terminate in sclerosis of the organ. Some are acute in character, others chronic and progressive. In the order of frequency they are as follows:

1. Alcoholic cirrhosis: about 50 per cent, is characterized by the presence of an acidophilic reticulum within the affected liver cells. A marked deposit of fat in the cells is a frequent complication, especially in the early stages. The islands of regeneration are usually not over 1 to 3 mm. in diameter. The lesion is not caused by ethyl alcohol, but probably by some toxic substance frequently contaminating liquors.

2. Pigment cirrhosis: about 9 per cent, is characterized by the presence of two yellow pigments, hemofuscin and hemosiderin, in the liver cells, in the bile duct epithelium, in the smooth muscle cells of the blood vessels, in fibroblasts, endothelial cells and macrophages. The islands of regeneration usually measure 1 to 3 mm. in diameter. The new liver cells contain no pigment at first, and they acquire hemofuscin granules which gradually change to hemosiderin granules. Slow necrosis of scattered liver cells is followed by regeneration. The lesion is probably due to copper which combined with hemoglobin is deposited as yellow pigment granules in the liver cells. The copper soon disappears and leaves behind hemofuscin which in time changes to hemosiderin.

3. Acute toxic cirrhosis: about 8 per cent, follows extensive acute necrosis, often involving all the liver cells in many lobules. The necrotic cells are dissolved and removed by the action of leukocytes, leaving large areas of red atrophy containing only sinusoids, stroma, proliferated bile ducts and blood vessels. The islands of regeneration are usually large, often 1 cm. or more in diameter. This type of lesion is due to a variety of toxic substances (phosphorus, arsenic, bacterial toxins).

4. Syphilitic cirrhosis: about 4 per cent, is due to *Treponema pallidum.* The lesion occurs as a diffuse sclerosis, especially in the congenital form, second-

ary to the action of the organisms on the stroma; and as infarcts (gummas) resulting from occlusion of blood vessels (the usual form of the lesion in acquired syphilis). Organization of infarcts leads to extensive scar formation and retraction (hepar lobatum).

5. Colon bacillus cirrhosis: about 4 per cent, is due to invasion of bile ducts (cholangitis), rarely of portal veins (pylephlebitis), by the colon bacillus. The lesion may be acute, recurrent, or continuous. It is located at the peripheries of the lobules, spreads towards the hepatic veins, and is often associated with obstruction of the common bile duct. Proliferation of small bile ducts is sometimes marked.

6. Obstructive cirrhosis: about 3 per cent, is due to occlusion of bile ducts, usually by calculus or cancer. The lesion is mechanical in origin. Obstruction causes distention of the bile ducts. The fibroblasts in the portal regions are stimulated to proliferate. The surface of the liver is finely granular. The liver cells often contain globules of bile which may become decolorized and then stain acidophilically.

Discussion

(Dr. Virgil H. Moon, Philadelphia, Pa.) In the first place, I wish to express my deep appreciation of the presentation.

One thing I should like Dr. Mallory to answer, if he will, is whether he regards the hyaline reticulum which is so characteristic of alcoholic cirrhosis as due to the alcohol as a factor, or whether it is simply the earmark of that type in which alcohol as an etiological factor may be absent. I had opportunity to perform an autopsy on one of three cases occurring in children in the same family, in which that form of hyaline material in many cells was very prominent. The familial occurrence was a remarkable feature. The fact that these children had had streptococcus infections a few years previous was a factor to be considered, and the absence of any actual or presumptive use of alcohol is also a factor to be considered.

(Dr. Mallory, closing.) Years ago a doctor passing through Boston showed me a section of the liver containing hyalin from a child in Philadelphia, and promised to send me a section to keep, but I never received it. I would appreciate it if you would send me a section or some of the tissue from your case for study.

I am sure ethyl alcohol does not cause alcoholic cirrhosis. The name may be poor, but for the present it has to be used. What we are working for is the etiological factor. If I had to guess as to the cause of alcoholic cirrhosis, I should say in the first place that it is something which contaminates alcohol. Secondly, we usually have, in combination with the hyaline reticulum, fatty infiltration of the cells. If there is one thing which may cause the two conditions, fatty infiltration and the hyaline change, it is possibly chronic arsenical poisoning, which might possibly occur in children in connection with something they have taken in their food or drink. That is just a suggestion. I have been working on the problem for thirty-three years, and cannot find the cause. But I am sure it is not alcohol.

STUDIES OF ABNORMAL LIVER FUNCTION. George H. Whipple, Rochester, N. Y. *Abstract.* Bile salts make up an important constituent of the bile and the experiments to be reported indicate that these salts are formed only through the activity of the liver. When the liver is injured, the output of these bile salts may

fall to very low levels, even with a slight injury of the liver. It is believed that bile is essential for normal existence, and animals deprived of it for weeks develop abnormal conditions leading shortly to death. It appears that bile salts are the most essential constituent of the bile and have many important interrelations within the body. Liver feeding may prevent certain types of intoxication in bile fistula dogs.

The second point is that the liver is essential to the construction of blood hemoglobin. Under carefully controlled conditions in anemia, the animal produces uniform amounts of new hemoglobin; when the liver is injured in certain special ways the output of hemoglobin under similar conditions may be decreased to 50 per cent normal. This indicates that the liver takes a part in the building up of hemoglobin in the body. Probably it helps in the assembling of amino acids to form the parent substances of hemoglobin utilized in the bone marrow, where the red cells are finished and turned out into the circulation. This observation is in harmony with the well known fact that the feeding of liver enables an animal or human being to make large amounts of new blood, and therefore explains the use of liver diet in anemia.

A Clinical and Pathological Study of 297 Cases of Cirrhosis of the Liver. James S. McCartney, Jr., Minneapolis, Minn.

Abstract. In this series of cases 87 per cent are of the portal or Laennec type, obstructive biliary and syphilitic cirrhoses making up the remainder. Of the portal type approximately two-thirds are considered as clinical and one-third as latent. Portal cirrhosis is more frequently latent in males than in females, whereas the clinical cases are about equally divided between the two sexes. The peak of incidence in the latent cases (30 per cent) is in the seventh decade; in the clinical cases it is in the sixth decade (27 per cent). Usually the livers from latent cases show, on microscopic examination, a less advanced process than is shown in the clinical cases, although an advanced cirrhosis is frequently present in the latent group. Small cirrhotic livers are much more frequently found in clinical cases than in the latent group, 25 per cent of the livers in the former group weighing 1000 gm. or less, as against 5 per cent in the latter. Livers weighing 2000 gm. or more are observed in about 25 per cent of each group. Spleens weighing 250 gm. or more are present in 66 per cent of clinical cases and in 39 per cent of latent cases.

Experimental Pigment Cirrhosis of the Liver Due to Copper Poisoning. E. M. Hall and (by invitation) E. M. MacKay, Los Angeles, Calif.

Abstract. Methods. Healthy rabbits were selected. For each rabbit to be fed on copper, a litter-mate of the same sex was selected as a control. Each series when 85 days old was placed on a special diet consisting of a mixture of ground alfalfa and ground barley. When 90 days of age, one series was placed on a diet in which 2 mg. of copper were added to each gram of the special diet. These diets and tap water were allowed *ad libitum*. Many of the copper-fed rabbits died after several weeks, due to copper poisoning, while none of the control rabbits died before being sacrificed.

Results. Seventeen out of twenty-one copper-fed rabbits showed pigmentation of the liver. The pigmentation consisted mainly of hemofuscin granules which were found closely packed in large Kupffer multinuclear giant cells. A few of the livers contained hemosiderin in small amounts. Ten out of twenty-

one rabbits showed cirrhosis of the liver. When we consider that five of the twenty-one rabbits were fed copper for less than 30 days, it is evident that the percentage of pigmentation and cirrhosis is relatively high in those animals fed for 30 days or more. In the control rabbits practically no copper was found in their livers, while in the copper-fed animals there was found from 9.68 mg. to 237 mg. per 100 gm. of wet liver tissue. The copper-fed animals showing no pigmentation or cirrhosis were animals showing less than 100 mg. of copper per 100 gm. of liver substance. All but one animal that received copper for more than 40 days showed pigmentation, which in most cases was very marked. All of the animals receiving copper for more than 60 days showed cirrhosis of the liver. The animals were fed on their special diets for periods ranging from 21 to 105 days.

Discussion

(Dr. Virgil H. Moon, Philadelphia.) I should like to ask if the authors of the paper have made an analysis, in human cases of hemochromatosis, for the presence of copper in the liver.

(Dr. Frank B. Mallory, Boston.) There has been a lot of work done in Aschoff's laboratory by Schönheimer and others. At first they were very enthusiastic about the presence of copper, but when they analyzed other cases of cirrhosis, they found an almost equal amount. I think the explanation is that the copper quickly disappears from the liver, and in these chronic cases it never has a chance to accumulate. It is not a cumulative poison, anyway. The copper disappears from its combination with hemoglobin, leaving hemofuscin. In two cases of very acute pigment cirrhosis with islands of regeneration, the islands, as I told you, at first contain no pigment, and then we get a pigment which gives a copper reaction. Fixed in formalin, the hemosiderin would not stain, except brown, while the copper stains blue. I have not gone far enough yet, but in these cases I get single cells and groups of cells which give a blue reaction (and that would signify that in the early stages copper was there), but in a few weeks it disappears.

(Dr. Hall, closing.) We have seen some of the acute changes pointed out by Dr. Mallory, and we agree with him in regard to them. Dr. Mallory has made it appear that it is extremely easy to demonstrate the presence of copper in these granules of hemofuscin. In 1928 I tried very hard to do this, for I thought there was copper in these granules, since the amounts of copper stored in the liver ran quite proportional to the amount of pigment present. Dr. Mallory has worked on this problem for ten years, and has only lately described a method for staining copper in the hemofuscin granules. I have also felt that there was more or less bound iron in the hemofuscin, and perhaps it may change to hemosiderin through oxidation. I have tried by means of incinerating the sections and oxidizing the bound iron to make it show up, but I think I lost it later in trying to stain it by the Berlin blue method. It might be possible to demonstrate it by Scott's method.

I wish to add that we fed three rabbits with their litter-mate controls on a diet containing sodium acetate for periods of 40 to 60 days, and we found no pigmentation. I am sure that this pigment that Flinn and VonGlahn found with sodium acetate is the natural pigment of the liver cells. We avoided carrots in our rabbits' diet, and we had none in our previous work, so it seems to me impossible that the pigmentation could be due to this factor of feeding carrots.

EXPERIMENTAL COPPER POISONING. Frank B. Mallory and Frederic Parker, Jr., Boston, Mass.

Abstract. Copper is best administered by injecting subcutaneously a suspension of metallic copper powder in lard. The acute lesions, occurring within the first two or three weeks, are anemia, acute tubular nephritis, central or diffuse necrosis of the liver, hemoglobinuria, pigmentation of the liver, and sometimes of the kidneys. The chronic lesions consist chiefly of pigmentation of the liver with accompanying necrosis of scattered liver cells, of sclerosis which starts at the peripheries of the lobules where the pigmentation is always most marked, and of regeneration of liver cells diffusely and in small islands.

The early pigment granules in the liver stain quickly a deep blue in a slightly alkaline, aqueous solution of pure hematoxylin after fixation in alcohol. In this way they may readily be distinguished from the yellow pigment granules which may occur, sometimes in abundance, under natural conditions, in the liver of the rabbit and which do not stain with hematoxylin; while hemosiderin stains yellow to brown.

The blue staining of the pigment granules is evidently due to the presence of copper which disappears in the course of a few months, leaving behind hemofuscin granules which gradually change to hemosiderin. Both hemofuscin and the pigment granules occurring under natural conditions can be stained by basic aniline dyes.

STUDIES IN EXPERIMENTAL CINCHOPHEN POISONING. T. P. Churchill and F. H. van Wagoner (by invitation), Chicago, Ill.

Abstract. Dogs have been fed cinchophen in doses ranging from that used therapeutically to twenty-seven times that amount. About 80 per cent of these animals have shown one or more acute gastric ulcers. Areas of necrosis have been found in the livers of the dogs that have died to date. The animals have refused to eat food containing the drug for more than a few days at a time. During the periods of taking the drug there occurred an increasing retention of bromsulphalin and a decrease in blood urea. After several days of refusal to eat any food, bromsulphalin was again excreted in normal amount and the urea content of the blood rose to normal. This work is still in progress.

Discussion

(Dr. Paul Klemperer, New York City.) Dr. Fishberg and I tried to produce liver changes in rabbits by feeding them Atophan, the Schering and Glatz preparation, and by giving it intravenously. Some of the animals died very shortly after the intravenous injection, so we could not expect them to show anything; other animals were given by mouth ten to twenty times the therapeutic dosage, and we were unable to find any definite change. I think Dr. Churchill's experiments also point in the same direction, that we must still be rather cautious in concluding that Atophan generally produces an acute liver atrophy. In the last three years we have observed three cases in which the clinicians were sure that the Atophan was the cause of subacute yellow atrophy. In these cases we did not see any gastric ulcer. We found marked duodenitis which is, however, often found in cases of acute yellow atrophy in which no Atophan was given.

(Dr. Howard T. Karsner, Cleveland.) I wish to make reference to some publications by Dr. H. S. Reichle, a former member of this Department, which dealt

with autopsies on patients who died of so-called toxic cirrhosis of the liver, in whose history there was the appearance of susceptibility to cinchophen.* I recall not less than three of these cases, and in none of them was there an ulcer found in the stomach. Dr. Reichle continued this work on experiments with rats, and administered cinchophen in massive doses over long periods of time without discovering any lesion in the liver. He then attempted to injure the liver by chloroform treatment, and we assumed from controls that that injury was produced. Even after that injury, the prolonged administration of cinchophen had no effect. I think that Dr. Reichle's experiments lead us to assume that, as far as the rat is concerned, there is no liver injury produced by cinchophen, and from a survey of the whole matter I am inclined to say that, as far as man is concerned, the damage by cinchophen represents an idiosyncrasy.

(Dr. Churchill, closing.) I recall seeing those three cases that Dr. Karsner had reference to. I was at the City Hospital at the time. I do not mean to give the impression that I do not think Atophan does not produce liver damage; and the purpose of the paper is to produce acute yellow atrophy in the dog. However, none of those areas of necrosis was observed grossly, enough to cause very definite areas of necrosis in the gross pathology. They exhibited small yellowish areas, and the microscopic findings were the only ones where we found areas of necrosis at all.

THE SPECIFIC CHARACTERS OF TOXIC CIRRHOSIS AS OBSERVED IN CINCHOPHEN POISONING. A REVIEW OF FIVE FATAL CASES. D. C. Beaver (by invitation), and H. E. Robertson, Rochester, Minn.

Abstract. Attention is called to certain specific characters which these forms of cirrhosis possess. Particular attention is given to the types produced by antirheumatic medication, especially the cinchophen groups of drugs. Of these latter five fatal cases are reported. Attempt is made to correlate these cases with other types of so-called toxic cirrhosis and to differentiate them from the so-called atrophic, or Laennec's cirrhosis.

Discussion

(Dr. Robertson.) It may be possible that the changes which have been described in this paper are not produced by cinchophen, and yet it is a remarkable fact that in several of these cases, when cinchophen was taken away from the patient, he became better and when it was given again he became worse and died. The lesions in these cases bear a certain resemblance to those of acute yellow atrophy, but their course is more chronic than acute yellow atrophy and still more rapid than the ordinary types of cirrhosis of the liver. Repair in these cases has more prominence than that of acute yellow atrophy, but less than the ordinary cirrhosis. There are all gradations, however, between the groups which we are accustomed to denominate acute yellow atrophy, cases of toxic cirrhosis such as those that are reported in this paper and which might be denominated subacute yellow atrophy, and the finished stages of so-called Laennec's or portal cirrhosis. I should like to be able to demonstrate that all of these types are related to a toxic destruction of the liver cells, but this hardly seems possible at the

* Reichle, H. S.: Toxic Cirrhosis of the Liver due to Cinchophen. *Arch. Int. Med.*, 1929, 44, 281.

present time. It may be quite true, however, that many of the acute yellow atrophies of the early days had as their etiological agent some of these drugs.

It is very true that there is marked idiosyncrasy exhibited by the patient in the production of this disease. The clinicians have repeatedly noted that many patients consume large quantities of cinchophen without the slightest evidence of harm. On the other hand, certain individuals apparently are severely poisoned by even small doses. When the poisoning comes, its course is very rapid and jaundice a very early characteristic.

STREPTOCOCCUS HEPATITIS. H. Edward MacMahon and Frank B. Mallory, Boston, Mass.

Abstract. The more common inflammatory changes seen in the liver in cases of streptococcus septicemia are described and illustrated with cases that have come to autopsy.

Emphasis, however, is laid on a less common lesion of which three cases are given in detail, characterized by rather sharply circumscribed and irregularly distributed areas of liver tissue showing necrobiotic changes and necrosis, infiltrated with polymorphonuclear and endothelial leucocytes and showing masses of streptococci both intra- and extracellularly. This lesion — one of acute hepatitis — is ascribed to the toxic action of streptococci acting locally within the area involved.

The similarity of this lesion to the histological picture at times encountered in cases of acute yellow atrophy is discussed, and the suggestion is made that a careful search of the liver in the fixed preparation for streptococci, together with culture of the liver at the time of autopsy, might reveal bacteria within the lesions more commonly than is suspected — particularly in those cases of so-called acute yellow atrophy showing a very irregular distribution of the lesion — a condition that is extremely difficult to explain on the basis of a circulating toxin in the blood.

A fourth case is fully described showing a chronic inflammatory reaction within the liver in which one finds a picture of degeneration, necrobiosis and necrosis of liver cells, with dissociation of the intercellular reticulum, together with an active proliferation of bile ducts and connective tissue and a rather rich infiltration of endothelial leucocytes and lymphocytes. These changes are irregularly scattered throughout the liver, and in places have entirely replaced many lobules. Streptococci are numerous and confined to the lesions. A non-hemolytic streptococcus was recovered from the patient's blood during life, and a similar organism was isolated in pure culture from the liver at the time of autopsy. This case is presented rather for discussion than as a proved case of progressive cirrhosis of infectious origin.

The last point to be considered is the histological and gross changes which one may find in the healed stages of these acute and chronic inflammatory processes, and here again the suggestion is offered that certain types of cirrhosis, usually diagnosed as healed acute yellow atrophy of an atypical type (lesions that have been described as isolated cirrhotic nodules within the liver) may represent healed infectious hepatitis of bacterial origin.

The second part of the paper is devoted to results of experimental work. A streptococcus obtained from an early case of scarlet fever was injected, free of toxin, into radicles of the portal veins of both guinea pigs and rabbits. The animals were killed at varying intervals and the lesions produced, together with

the results of bacteriological studies, are described and compared with the lesions seen in human cases.

It should be pointed out very clearly that absolutely no attempt has been made to explain cirrhosis of the liver in its broad sense as a chronic or healed inflammatory process of infectious origin. On the contrary, the points that are particularly emphasized are just that an acute inflammation can occur in the liver as a result of the actual presence of streptococci within this organ; second, that with extensive destruction of liver cells, and the reparative proliferation of connective tissue and bile ducts, wide tracts of sclerosis can be produced — giving a picture which resembles in many respects the healed stages of acute yellow atrophy.

Discussion

(Dr. Stuart Mudd, Villa Nova, Pa.) I should like to ask if this finding of phagocytosis by the endothelial leucocytes rather than by the polymorphonuclears is in general characteristic of streptococcus hepatitis.

(Dr. MacMahon.) In the first case reported the reacting cells were almost entirely endothelial leucocytes. In other cases also showing the acute lesions, polymorphonuclear leucocytes were equally as numerous. In answer to Dr. Mudd's question, in our cases phagocytosis of the streptococci has been limited to the endothelial leucocytes.

(Dr. Alfred Plaut, New York City.) In the cases reported a focus could be demonstrated as the source of infection. In case an infant lived for a longer time, it might happen that this demonstration would be no longer possible. Only two weeks ago I did an autopsy on an infant 30 days old. I found streptococcus peritonitis, and an enlarged liver which in the gross looked exactly like the liver of congenital syphilis — so much so that I started to hunt for spirochetes in spite of the absence of any other gross signs. No spirochetes were found. The peritoneum gave a pure culture of streptococcus. The liver microscopically contained a meshwork of necrosis which went irregularly through the whole liver. The umbilical vessels, examined in cross-section from five different planes, were entirely normal, the degree of occlusion corresponding to the age of the infant. Later on I saw the physician who had seen the infant before it came to the hospital, and he told me the child had had a typical septic infection of the umbilicus. I wonder what might happen to a liver if an infant should survive such an infection.

(Dr. MacMahon.) This is the type of case we are primarily interested in, one showing an acute destructive inflammatory lesion of the parenchyma. It would be interesting if streptococci could be demonstrated within the more recent lesions of this liver. Even if organisms could not be demonstrated, streptococci should not be definitely excluded, because streptococci acting locally can produce considerable damage, and in a very short time may be killed and completely removed.

I have not reviewed the literature regarding the changes that one would find in the liver had the child recovered, but I have found cases reported showing isolated foci of cirrhosis of unidentified origin. We have also seen livers both in children and in adults showing isolated patches of cirrhosis. Some of these cases may be explained on the assumption that at one time there was an infectious lesion which had subsided.

(Dr. Preston Kyes, Chicago.) Some fifteen years ago I published a paper, the conclusion of which was that the resistance of birds to pneumococci was due to

present time. It may be quite true, however, that many of the acute yellow atrophies of the early days had as their etiological agent some of these drugs.

It is very true that there is marked idiosyncrasy exhibited by the patient in the production of this disease. The clinicians have repeatedly noted that many patients consume large quantities of cinchophen without the slightest evidence of harm. On the other hand, certain individuals apparently are severely poisoned by even small doses. When the poisoning comes, its course is very rapid and jaundice a very early characteristic.

STREPTOCOCCUS HEPATITIS. H. Edward MacMahon and Frank B. Mallory, Boston, Mass.

Abstract. The more common inflammatory changes seen in the liver in cases of streptococcus septicemia are described and illustrated with cases that have come to autopsy.

Emphasis, however, is laid on a less common lesion of which three cases are given in detail, characterized by rather sharply circumscribed and irregularly distributed areas of liver tissue showing necrobiotic changes and necrosis, infiltrated with polymorphonuclear and endothelial leucocytes and showing masses of streptococci both intra- and extracellularly. This lesion — one of acute hepatitis — is ascribed to the toxic action of streptococci acting locally within the area involved.

The similarity of this lesion to the histological picture at times encountered in cases of acute yellow atrophy is discussed, and the suggestion is made that a careful search of the liver in the fixed preparation for streptococci, together with culture of the liver at the time of autopsy, might reveal bacteria within the lesions more commonly than is suspected — particularly in those cases of so-called acute yellow atrophy showing a very irregular distribution of the lesion — a condition that is extremely difficult to explain on the basis of a circulating toxin in the blood.

A fourth case is fully described showing a chronic inflammatory reaction within the liver in which one finds a picture of degeneration, necrobiosis and necrosis of liver cells, with dissociation of the intercellular reticulum, together with an active proliferation of bile ducts and connective tissue and a rather rich infiltration of endothelial leucocytes and lymphocytes. These changes are irregularly scattered throughout the liver, and in places have entirely replaced many lobules. Streptococci are numerous and confined to the lesions. A non-hemolytic streptococcus was recovered from the patient's blood during life, and a similar organism was isolated in pure culture from the liver at the time of autopsy. This case is presented rather for discussion than as a proved case of progressive cirrhosis of infectious origin.

The last point to be considered is the histological and gross changes which one may find in the healed stages of these acute and chronic inflammatory processes, and here again the suggestion is offered that certain types of cirrhosis, usually diagnosed as healed acute yellow atrophy of an atypical type (lesions that have been described as isolated cirrhotic nodules within the liver) may represent healed infectious hepatitis of bacterial origin.

The second part of the paper is devoted to results of experimental work. A streptococcus obtained from an early case of scarlet fever was injected, free of toxin, into radicles of the portal veins of both guinea pigs and rabbits. The animals were killed at varying intervals and the lesions produced, together with

the results of bacteriological studies, are described and compared with the lesions seen in human cases.

It should be pointed out very clearly that absolutely no attempt has been made to explain cirrhosis of the liver in its broad sense as a chronic or healed inflammatory process of infectious origin. On the contrary, the points that are particularly emphasized are just that an acute inflammation can occur in the liver as a result of the actual presence of streptococci within this organ; second, that with extensive destruction of liver cells, and the reparative proliferation of connective tissue and bile ducts, wide tracts of sclerosis can be produced — giving a picture which resembles in many respects the healed stages of acute yellow atrophy.

Discussion

(Dr. Stuart Mudd, Villa Nova, Pa.) I should like to ask if this finding of phagocytosis by the endothelial leucocytes rather than by the polymorphonuclears is in general characteristic of streptococcus hepatitis.

(Dr. MacMahon.) In the first case reported the reacting cells were almost entirely endothelial leucocytes. In other cases also showing the acute lesions, polymorphonuclear leucocytes were equally as numerous. In answer to Dr. Mudd's question, in our cases phagocytosis of the streptococci has been limited to the endothelial leucocytes.

(Dr. Alfred Plaut, New York City.) In the cases reported a focus could be demonstrated as the source of infection. In case an infant lived for a longer time, it might happen that this demonstration would be no longer possible. Only two weeks ago I did an autopsy on an infant 30 days old. I found streptococcus peritonitis, and an enlarged liver which in the gross looked exactly like the liver of congenital syphilis — so much so that I started to hunt for spirochetes in spite of the absence of any other gross signs. No spirochetes were found. The peritoneum gave a pure culture of streptococcus. The liver microscopically contained a meshwork of necrosis which went irregularly through the whole liver. The umbilical vessels, examined in cross-section from five different planes, were entirely normal, the degree of occlusion corresponding to the age of the infant. Later on I saw the physician who had seen the infant before it came to the hospital, and he told me the child had had a typical septic infection of the umbilicus. I wonder what might happen to a liver if an infant should survive such an infection.

(Dr. MacMahon.) This is the type of case we are primarily interested in, one showing an acute destructive inflammatory lesion of the parenchyma. It would be interesting if streptococci could be demonstrated within the more recent lesions of this liver. Even if organisms could not be demonstrated, streptococci should not be definitely excluded, because streptococci acting locally can produce considerable damage, and in a very short time may be killed and completely removed.

I have not reviewed the literature regarding the changes that one would find in the liver had the child recovered, but I have found cases reported showing isolated foci of cirrhosis of unidentified origin. We have also seen livers both in children and in adults showing isolated patches of cirrhosis. Some of these cases may be explained on the assumption that at one time there was an infectious lesion which had subsided.

(Dr. Preston Kyes, Chicago.) Some fifteen years ago I published a paper, the conclusion of which was that the resistance of birds to pneumococci was due to

the very rapid destruction of these organisms by the fixed tissue phagocytes of the sinusoids of the liver. In such experiments as were done and reported in that article, I showed that the pneumococci were completely destroyed in 112 hours. The phagocytic cells do not infiltrate the capillaries, but are actual constituent cells of the capillary walls. These cells may be desquamated after they have phagocyted the pneumococci.

(Dr. Virgil H. Moon, Philadelphia.) I am interested in the presentation of this work, particularly as it duplicates some of the features that came under my observation not so long ago in a juvenile case of cirrhosis, having the gross features of Banti's disease or atrophic cirrhosis, in which cultures were made as described by Dr. MacMahon. A hemolytic streptococcus was obtained in pure culture from the substance of the liver. It was not obtained from postmortem blood culture, or from the blood during life. The same organism was obtained from the spleen. It was not found in the kidney. These facts indicate that it was not a terminal septicemia. Another case of similar character in which we had no opportunity to make cultures showed identical microscopic demonstration of the organism in the substance of the liver and of the spleen. An adult case of atrophic cirrhosis came to autopsy a few weeks ago. A pure culture of hemolytic streptococcus was obtained from the liver substance. The pathogenicity of this streptococcus is being studied at this time.

(Dr. MacMahon, closing.) I have been very much interested in the excellent work on infectious lesions of the liver already done by Dr. Moon.

Both cases which he has reported show extensive and long standing cirrhosis, and in both streptococci have been demonstrated; however, it is rather difficult to be sure exactly what part the streptococci are playing in such lesions of the liver.

In résumé, we believe that streptococci may produce an acute destructive inflammatory reaction within the parenchyma of the liver; that the organisms may be rapidly destroyed and removed from the areas of inflammation; and lastly there is evidence to show that the smaller foci may completely heal, whereas the larger areas probably result in isolated patches of cirrhosis.

GRANULOMATOUS ABSCESS OF THE LIVER OF PYOGENIC ORIGIN. D. C. Beaver (by invitation), Rochester, Minn.

Abstract. This is a study of eight cases of chronic pyogenic granulomatous infections of the liver which have a fairly distinct clinical and pathological picture. Their resemblance to actinomycotic infections of the liver is remarkable. Comparisons with actinomycosis and with more acute pyogenic abscesses of the liver will be presented.

CHANGES INDUCED IN THE LIVER BY MECHANICAL OBSTRUCTION OF THE HEPATIC VEINS. J. P. Simonds and (by invitation) J. W. Callaway, Chicago, Ill.

Abstract. The hepatic veins of dogs were mechanically obstructed by clamping off the hepatic veins by the method previously described by Simonds and Brandes. Careful asepsis was observed in these experiments and the animals were allowed to live for from 48 to 72 hours and then sacrificed. The following changes were observed in the livers of these animals: (1) scattered areas of marked passive hyperemia with disruption of the cords of liver cells; (2) edema and hemorrhage in the connective tissue about the sublobular veins; (3) areas of focal necrosis; (4) swelling and granulation of the cells in the central portions

of the lobules; (5) phagocytosis of erythrocytes and excessive hemosiderin pigmentation of Kupffer cells; (6) hyaline thrombi in many central and sublobular veins, and (7) the presence of masses of cells occluding the sinusoids similar to the masses observed in dogs that have survived anaphylactic and peptone shock.

READ BY TITLE

ASSIMILATION OF ATLAS AND COMPRESSION OF MEDULLA: WITH CONTRIBUTION TO THE CLINICAL SIGNIFICANCE AND PATHOLOGY OF TORTICOLLIS AND LOCALIZED CHRONIC ARTHRITIS DEFORMANS OF THE SPINE. Istvan Bezi (by invitation), Budapest and Rochester, N. Y.

STUDIES ON THE ADSORPTION OF DIPHTHERIA TOXIN ONTO AND ELUTION FROM MAGNESIUM HYDROXIDE. E. E. Ecker and (by invitation) L. A. Weed, Cleveland, O.

AN INVESTIGATION OF SPIROCHETOSIS OF THE DENTAL ANLAGE IN CONGENITAL SYPHILIS. T. J. Hill (by invitation), Cleveland, O.

THREE FATAL CASES OF BACILLUS PYOCYANEUS INFECTION. B. S. Kline and (by invitation) A. S. Maschke, Cleveland, O.

THE PATHOLOGY OF BARTONELLA MURIS ANEMIA AND THE COMPENSATORY PHENOMENA FOLLOWING SPLENECTOMY IN THE ALBINO RAT. J. Marmorston-Gottesman and David Perla, New York City.

ACUTE ENCEPHALOMYELITIS OF THE HORSE. EPIZOÖTIC IN THE SAN JOAQUIN VALLEY, 1930. K. F. Meyer, San Francisco, Calif.

URINE FLOW AND OPEN GLOMERULI AFTER UNILATERAL NEPHRECTOMY. R. A. Moore and (by invitation) W. W. Summerville, Cleveland, O.

X-RAY SARCOMA OF MAN WITH REPORT OF A CASE. Frederick W. Mulsow, Cedar Rapids, Iowa.

MYOCARDIUM IN SYPHILIS. Otto Saphir, Chicago, Ill.